



INVESTOR IN PEOPLE

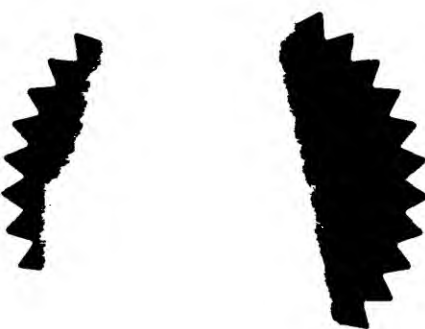
The Patent Office
Concept House
Cardiff Road
Newport
South Wales
NP10 8QQ

I, the undersigned, being an officer duly authorised in accordance with Section 74(1) and (4) of the Deregulation & Contracting Out Act 1994, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.



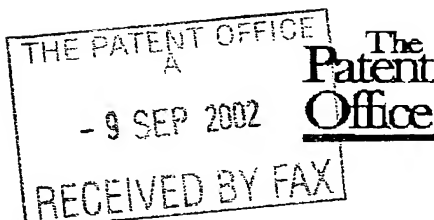
Signed

Andrew

Dated

15 August 2003

Patents Form 1/77

Patents Act 1977
(Rule 16)The
Patent
Office09SEP02 E746525-2 D02973
P01/7700 0.00-0220764.5

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
South Wales
NP9 1RH

1. Your reference

JHC/P041070GB1

2. Patent application number
(The Patent Office will fill in this part)

0220764.5

- 9 SEP 2002

3. Full name, address and postcode of the or of each applicant (underline all surnames)

Trigen Limited
20 St James's Street
LONDON
SW1A 1ES

Patents ADP number (if you know it)

7516081001

If the applicant is a corporate body, give the country/state of its incorporation

United Kingdom

4. Title of the invention

Borozeptides

5. Name of your agent (if you have one)

Harrison Goddard Foote

"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)

Belgrave Hall
Belgrave Street
Leeds
LS2 8DD

Patents ADP number (if you know it)

14571001-7631310002

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number

Country

Priority application number
(if you know it)Date of filing
(day / month / year)

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application

Date of filing
(day / month / year)

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if:

a) any applicant named in part 3 is not an inventor, or

b) there is an inventor who is not named as an applicant, or

c) any named applicant is a corporate body.

See note (d))

Yes

Patents Form 1/77

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form

Description	51 ✓ m
Claim(s)	9 ✓
Abstract	—
Drawing(s)	—

10. If you are also filing any of the following, state how many against each item.

Priority documents

Translations of priority documents

Statement of inventorship and right to grant of a patent (Patents Form 7/77)

Request for preliminary examination and search (Patents Form 9/77)

Request for substantive examination (Patents Form 10/77)

Any other documents (please specify)

11. I/we request the grant of a patent on the basis of this application.

Signature

Date

9 September 2002

12. Name and daytime telephone number of person to contact in the United Kingdom

Jonathan Couchman

0113 233 0100

Warning

After an application for a patent has been filed, the Comptroller of the Patent Office will consider whether publication or communication of the invention should be prohibited or restricted under Section 22 of the Patents Act 1977. You will be informed if it is necessary to prohibit or restrict your invention in this way. Furthermore, if you live in the United Kingdom, Section 23 of the Patents Act 1977 stops you from applying for a patent abroad without first getting written permission from the Patent Office unless an application has been filed at least 6 weeks beforehand in the United Kingdom for a patent for the same invention and either no direction prohibiting publication or communication has been given, or any such direction has been revoked.

Notes

- If you need help to fill in this form or you have any questions, please contact the Patent Office on 0645 500505.
- Write your answers in capital letters using black ink or you may type them.
- If there is not enough space for all the relevant details on any part of this form, please continue on a separate sheet of paper and write "see continuation sheet" in the relevant part(s). Any continuation sheet should be attached to this form.
- If you have answered 'Yes' Patents Form 7/77 will need to be filed.
- Once you have filled in the form you must remember to sign and date it.
- For details of the fee and ways to pay please contact the Patent Office.

Patents Form 1/77

P41070GB1.2 - 50c Salts/Generic Salts I

1

TITLE OF THE INVENTION

BOROPEPTIDES

BACKGROUND OF THE INVENTION

5

The present invention relates to boro-peptides useful in the treatment of thrombosis. More particularly, it relates to such boro-peptides of which one application is oral pharmaceutical formulations. In one aspect, it relates to the improvement of the oral administration properties of boronate esters.

10

Antithrombotic Drugs

Haemostasis is the normal physiological process in which bleeding from an injured blood vessel is arrested. It is a dynamic and complex process in which proteolytic enzymes such as thrombin play a key role. Blood coagulation may occur through either of two cascades of zymogen activations, the extrinsic and intrinsic pathways of the coagulation cascade. Factor VIIa in the extrinsic pathway, and Factor IXa in the intrinsic pathway are important determinants of the activation of factor X to factor Xa, which itself catalyses the activation of prothrombin to thrombin. The last protease in each pathway is thrombin, which acts to hydrolyse four small peptides (two FpA and two FpB) from each molecule of fibrinogen, thus deprotecting its polymerisation sites. Once formed, the linear fibrin polymers may be cross-linked by factor XIIIa, which is itself activated by thrombin. In addition, thrombin is a potent activator of platelets, upon which it acts at specific receptors. Thrombin activation of platelets leads to aggregation of the cells and secretion of additional factors that further accelerate the creation of a haemostatic plug. Thrombin also potentiates its own production by the activation of factors V and VIII (see Hemker and Beguin in: Jolles, et. al., "Biology and Pathology of Platelet Vessel Wall Interactions," pp. 219-26 (1986), Crawford and Scrutton in: Bloom and Thomas, "Haemostasis and Thrombosis," pp. 47-77, (1987), Bevers, et. al., *Eur. J. Biochem.* **1982**, 122, 429-36, Mann, *Trends Biochem. Sci.* **1987**, 12, 229-33).

30

A thrombus can be considered as an abnormal product of a normal mechanism and can be defined as a mass or deposit formed from blood constituents on a surface of the cardiovascular system, for example of the heart or a blood vessel. Thrombosis can be regarded as the pathological condition wherein improper activity of the haemostatic mechanism results in intravascular thrombus formation.

35

The management of thrombosis commonly involves the use of thrombolytic agents in combination with anticoagulants and antiplatelet drugs (inhibitors of platelet aggregation) to lyse

P41070GB1.2 - 50c Salts/Generic Salts I

2

the newly formed clot and to control future thrombogenesis. Anticoagulants are used also in the treatment of patients thought susceptible to thrombosis.

Currently, two of the most effective classes of drugs in clinical use as anticoagulants are the heparins and the vitamin K antagonists. The heparins are ill-defined mixtures of sulfated polysaccharides that bind to, and thus potentiate the action of antithrombin III. Antithrombin III is a naturally occurring inhibitor of the activated clotting factors IXa, Xa, XIa, thrombin and probably XIIa (see Jaques, *Pharmacol. Rev.* **1980**, 31, pp. 99-166). The vitamin K antagonists, of which warfarin is the most well-known example, act indirectly by inhibiting the post-ribosomal carboxylations of the vitamin K dependent coagulation factors II, VII, IX and X (see Hirsch, *Semin. Thromb. Haemostasis* **1986**, 12, 1-11). While effective therapies for the treatment of thrombosis, heparins and vitamin K antagonists have the unfortunate side effects of bleeding, heparin-induced thrombocytopenia (in the case of heparin) and marked interpatient variability, resulting in a small and unpredictable therapeutic safety margin. The use of direct acting inhibitors of thrombin and other serine protease enzymes of the coagulation system is expected to alleviate these problems.

Boro-peptide Serine Protease Inhibitors

A wide variety of direct acting serine protease inhibitors have been tested, including boro-peptides, i.e. peptides containing a boronic acid analogue of an N-acyl- α -amino acid. Shenvi (EP-A-145441 and US 4499082) disclosed that certain peptides containing an α -aminoboronic acid were effective inhibitors of elastase and has been followed by numerous patent publications relating to boro-peptide inhibitors of serine proteases.

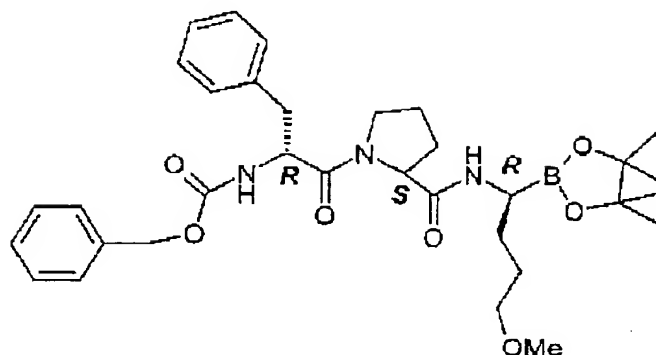
25

Claeson et al (US 5574014 and others) and Kakkar et al (WO 92/07869 and family members including US 5648338) disclose thrombin inhibitors having a neutral C-terminal side chain, for example an alkoxyalkyl side chain. The Claeson et al and Kakkar et al patent families disclose boronate esters containing the amino acid sequence D-Phe-Pro-BoroMpg [(R)-Phe-Pro-BoroMpg], which are highly specific inhibitors of thrombin (Deadman et al., *J. Med. Chem.* **1995**, 38, 1511-1522; Elgendy et al *Adv. Exp. Med. Biol. (USA)* **1993**, 340, 173-178). Of these compounds may be mentioned in particular Cbz-(R)-Phe-Pro-BoroMpg-OPinacol (also known as TRI50b).

30

The tripeptide sequence of TRI50b has three chiral centres. The Phe residue is considered to be of R (= D) configuration and the Pro residue of natural S (= L) configuration, at least in compounds with commercially useful inhibitor activity; the Mpg residue is believed to be of R (= L) configuration in isomers with commercially useful inhibitor activity. Thus, the most effective TRI50b stereoisomer is considered to be of RSR configuration and may be represented as:

35



(RSR)-TRI50b: Cbz-(R)-Phe-(S)-Pro-(R)-boroMpg Pinacol

- 5 The aforementioned US patents (US 5574014 and US 5648338) are incorporated herein by reference.

Oral Absorption

- 10 Absorption in the gastro-intestinal tract can be by an active or a passive route. Active absorption by transport mechanisms tends to be variable between individuals and with intestinal content (Gustaffson, D Thromb.Res., 2001, 101, 171-181). The upper intestine has been identified as the principal site of oral drug absorption. In particular, the duodenum is the customary target site for absorption of orally administered drugs because of its large surface area. The intestinal
- 15 mucosa acts as a barrier that controls passive transcellular absorption: the absorption of ionic species is blocked whilst the transcellular absorption of lipophilic molecules is favoured (Palm K et al., J.Pharmacol and Exp. Therapeutics, 1999, 291,435-443).

- 20 Orally administered drugs are required to be consistently and adequately absorbed. Variability of absorption between individuals or between different occasions in the same individual is unwelcome. Similarly, drugs which have a low level of bioavailability (only a small portion of the administered active agent is absorbed) are generally unacceptable.

- 25 Non-ionised compounds are favoured for passive absorption, a route associated with invariability, and are therefore preferred for consistent absorption. Lipophilic species are particularly favoured by passive absorption mechanisms and, accordingly, non-ionic, lipophilic drugs are indicated to be most favoured for consistent and high oral absorption.

P41070GB1.2 - 50c Salts/Generic Salts I

4

Typical functionalities required for interaction of drugs with their physiological targets are functional groups such as carboxylic and sulphonic acids. These groups exist as the protonated form in the stomach (at pH 2-3), but will be ionised to some extent at the higher pH of the intestinal fluid. One strategy that has been used to avoid the ionisation of the carboxylates or sulphonates is to present them as ester forms, which are cleaved once absorbed into the vascular lumen.

Oral Absorption of Boro-peptides

The boronate ester group of TRI50b is rapidly cleaved in the conditions of the plasma to form the corresponding boronic acid group, which is considered to be the active moiety which inhibits the catalytic site of thrombin.

Boronic acids are divalent functional groups, with boron-oxygen bond lengths (1.6Å) more typical of single bonds, unlike superficially comparable C-O and S-O bonds in carboxylic and sulphonic acids. Consequently the boronic acid group has two ionisation potentials. The boronic acid group will be partly ionised at pH's of the duodenal fluid and not suited to the desired passive duodenal uptake. Thus, a charged boronate inhibitor H-D-PheProBoroArg is absorbed by a predominantly active transport mechanism (Saitoh, H. and Aungst, B.J., Pharm. Res., 1999, 16, 1786-1789).

20

The peptide boronic acid formed by such cleavage of TRI50b is relatively insoluble in water, especially at acidic or neutral pH, and tends to be poorly absorbed in the stomach and duodenum. The acid has the structure Cbz-Phe-Pro-BoroMpg-OH.

Whereas the peptide boronic acid Cbz-Phe-Pro-BoroMpg-OH is partly ionised under duodenal conditions and, to that extent, unfavoured for passive transport, esters of the acid are designed for a high rate of passive (thus consistent) transport. The tripeptide sequence Phe-Pro-Mpg belongs to an unusual class of serine protease inhibitory peptide sequences in having a non-basic P1 side chain (specifically, methoxypropyl), such that the tripeptide consists of three non-polar amino acids. The esters of the peptide boronic acid are non-ionisable and the ester-forming species further impart lipophilic properties, so encouraging a high rate of passive transport.

Computational techniques have confirmed that TRI50b and other diol esters of Cbz-Phe-Pro-BoroMpg-OH can be predicted to have good bioavailability. Thus, polar surface area (PSAd) is a parameter predictive of bioavailability and PSAd values of greater than 60Å correlate well with passive transcellular transport and with bioavailability of known drugs (Kelder, J. Pharm. Res., 1999, 16, 1514-1519). Measurements for diol esters of the above peptide boronic acid, including

P41070GB1.2 - 50c Salts/Generic Salts I

5

the pinacol ester TRI50b, show that the diol esters have PSAd values well above 60Å, predictive of passive transport and good bioavailability as shown in Table 1:

Table 1: PSAd values of selected diol esters of Cbz-Phe-Pro-BoroMpg-OH

Diol	PSAd Value
Pinacol	98.74
Pinanediol	90.64

5

The corresponding monohydroxy alcohol (e.g. alkoxy) esters were considered too unstable, spontaneously cleaving to liberate the acid *in-vitro*. Esters of diols such as pinanediol and pinacol have enhanced kinetic stability over esters of monohydroxy alcohols, in that after partial hydrolysis to the mono-ester derivative they will tend to reassociate by a facile intra-molecular reaction.

10

To counterbalance these highly desirable features of TRI50b, it has been discovered that TRI50b tends to hydrolyse in acid media. Thus in the acid conditions of an HPLC assay, TRI50b is converted to the acid form with a short half life, which implies potential intraduodenal hydrolysis into ionic species which would resist passive transport and, if anything, be absorbed by active transport, indicative at best of variable bioavailability.

15

The instability of TRI50b to hydrolysis also presents potential disadvantages in preparation of the compound and its formulation, as well as in the storage of pharmaceutical formulations containing it.

20

An ideal solution to the instability of TRI50b would be development of a diol ester more stable to hydrolysis. In this regard, it is known that ring size can affect boronate stability and glycolato boron has been shown to have enhanced aqueous stability compared to pinacol (D.S.Matteson, Stereodirected Synthesis with Organoboranes, Springer-Verlag, 1995, ch.1). Similarly, the pinanediol ester is more stable than the pinacol; this is believed to be because the pinanediol group is highly sterically hindered and disfavours nucleophilic attack on the boron. In fact transesterification from pinacol to pinanediol has been reported (Brosz, CS, Tet. Assym, 1997, 8, 1435-1440) whereas the reverse process is unfavourable. The pinanediol ester however is considered too slow to cleave in plasma and there remains a need to provide an improved diol ester.

25

30

The properties described above will be shared by similar hydrophobic, non-basic boropeptides.

The present invention provides a different solution to the problem of boronate diol ester and especially TRI50b instability.

35

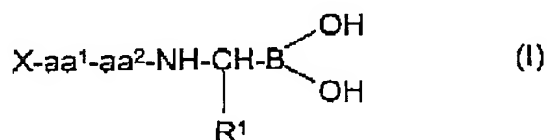
P41070GB1.2 - 50c Salts/Generic Salts I

6

BRIEF SUMMARY OF THE INVENTION

The invention provides a peptide boronic acid derivative which, in the conditions of the duodenum, contradictorily releases an ionic boropeptide species whilst avoiding the disadvantages of pinacol esters as well as enabling consistent and adequate bioavailability.

The present invention provides a salt of a peptide boronic acid of formula (I):



10

where:

X is H (to form NH₂) or an amino-protecting group;

15 aa¹ is Phe, Dpa or a wholly or partially hydrogenated analogue thereof;

aa² is an imino acid having from 4 to 6 ring members; and

R¹ is a group of the formula -(CH₂)_m-W, where m is 2, 3 or 4 and W is -OH, -OMe, -OEt or halogen (F, Cl, Br or I).

20

These salts are stable to hydrolysis and more soluble than corresponding peptide boronic acids. Their solubility and stability are unexpectedly indicated to more than offset the loss of a high passive absorption rate of the corresponding diol esters in terms of providing consistent and adequate bioavailability.

25

There is a debate in the literature as to whether boronates in aqueous solution form the 'trigonal' B(OH)₂ or 'tetrahedral' B(OH)₃⁻ boron species, but NMR evidence seems to indicate that at a pH below the first pKa of the boronic acid the main boron species is the neutral B(OH)₂. In the duodenum the pH is likely to be between 6 and 7, so the trigonal species is likely to be predominant here. In any event, the symbol -B(OH)₂ includes tetrahedral as well as trigonal boron species.

30

The invention includes also oral formulations of the salts of the invention.

P41070GB1.2 - 50c Salts/Generic Salts I

7

According to a further aspect of the present invention, there is provided a method of treatment of a condition where inhibition of thrombin is required which method comprises oral administration of a therapeutically effective amount of a salt of the invention to a person suffering from, or susceptible to, such a condition.

The salts of the invention are obtainable by reaction of the acid (I) with a strong base. The invention thus provides also products obtainable by reaction of an acid (I) with a base able to form a salt with it and the therapeutic, including prophylactic, use of such products.

10

The invention includes a method for preparing the salts from the corresponding peptide boronic acid as an intermediate, as well as the intermediate peptide boronic acid and a method for preparing it.

Further aspects and embodiments of the invention are set forth in the following description and claims.

Throughout the description and claims of this specification, the words "comprise" and "contain" and variations of the words, for example "comprising" and "comprises", mean "including but not limited to", and are not intended to (and do not) exclude other moieties, additives, components, integers or steps.

DETAILED DESCRIPTION OF THE INVENTION

25 Glossary

The following terms and abbreviations are used in this specification:

α -Aminoboronic acid or Boro(aa) refers to an amino acid in which the CO₂ group has been replaced by BO₂

Arg – arginine

Cbz – benzyloxycarbonyl

Cha – cyclohexylalanine

Dcha – dicyclohexylalanine

35 Dpa – diphenylalanine

Lys – lysine

Mpg – 3-methoxypropylglycyl

P41070GB1.2 - 50c Salts/Generic Salts I

8

Phe - phenylalanine

Pinac = Pinacol - 2,3-dimethyl-2,3-butanediol

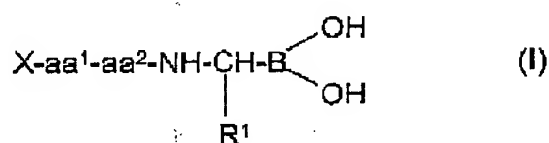
(+) - Pinanediol boronate - 1a,7,7-trimethyl-[1aS-{1aa, 4a, 6a, 5aa}] - 4,6-methano-1,2-benzodioxaborole

5 Pro - proline

Thr - thrombin

The Compounds

10 The products of the invention comprise salts of a peptide boronic acid of formula (I):

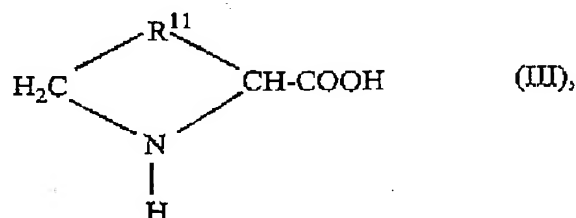


X is a moiety bonded to the N-terminal amino group and may be H to form NH₂. The identity of X is not critical to the invention but as a preferred example there may be mentioned

15 benzyloxycarbonyl.

aa¹ is Phe, Dpa or a wholly or partially hydrogenated analogue thereof. The hydrogenated analogues are Cha and D-Dcha.

20 A preferred class of products comprises those in which aa² is a residue of an imino acid of formula (III)



where R¹¹ is -CH₂-, CH₂-CH₂-, -S-CH₂-, -S-C(CH₃)₂- or -CH₂-CH₂-CH₂-, which group when the ring is 5 or 6-membered is optionally substituted at one or more -CH₂- groups by from 1 to 3 C₁-C₃ alkyl groups. Of these, azetidine-2-carboxylic acid, especially (S)-azetidine-2-carboxylic acid, and more particularly proline are preferred.

25

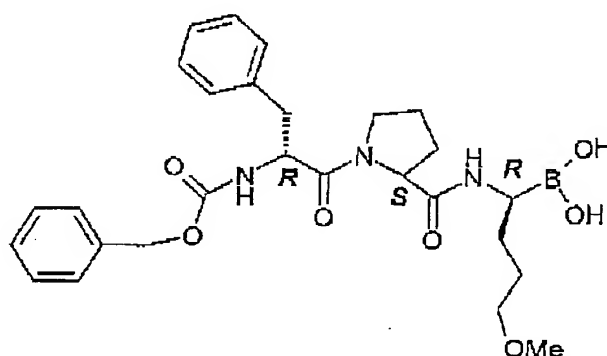
It will be appreciated from the above that a very preferred class of products consists of those in which aa¹-aa² is Phe-Pro. In another preferred class, aa¹-aa² is Dpa-Pro. In other products, aa¹-aa² is Cha-Pro or Dcha-Pro. Of course, the invention includes corresponding product classes in which Pro is replaced by (s)-azetidine-2-carboxylic acid,

5

R¹ is a group of the formula -(CH₂)_m-W. Integer m is 2, 3 or 4 and W is -OH, -OMe, -OEt or halogen (F, Cl, I or, preferably, Br). The most preferred W groups are -OMe and -OEt, especially -OMe. It is preferred that m is 3 for all W groups and, indeed, for all compounds of the invention. Particularly preferred R¹ groups are 2-bromoethyl, 2-chloroethyl, 2-methoxyethyl, 4-bromoethyl, 4-chloroethyl, 4-methoxyethyl and, especially, 3-bromopropyl, 3-chloropropyl and 3-methoxypropyl. Most preferably, R¹ is 3-methoxypropyl. 2-Ethoxyethyl is another preferred R¹ group.

Accordingly, a very preferred class of salts consists of those of acids of the formula X-Phe-Pro-Mpg-B(OH)₂, especially Cbz-Phe-Pro-Mpg-B(OH)₂; also preferred are analogues of these compounds in which Mpg is replaced by a residue with another of the particularly preferred R¹ groups and/or Phe is replaced by Dpa or another aa¹ residue.

The aa¹ moiety of the salt is preferably of R configuration (D-configuration). The aa² moiety is preferably of S configuration (L-configuration). Particularly preferred salts have aa¹ of R configuration and aa² of S configuration. The chiral centre -NH-CH(R¹)-B- is preferably of R configuration. It is considered that commercial formulations will have the chiral centres in RSR arrangement, as for example in the case of salts of Cbz-Phe-Pro-BoroMpg-OH:



Cbz-(R)-Phe-(S)-Pro-(R)-boroMpg-OH

The salts are obtainable by contacting an acid of formula (I) with a strong base. The invention thus contemplates products (compositions of matter) having the characteristics of a reaction product of an acid of formula (I) and a strong base. The base is pharmaceutically acceptable.

P41070GB1.2 - 50c Salts/Generic Salts I

10

As suitable salts may be mentioned:

1. Alkali metal salts;
2. Alkaline earth metal salts;
3. Group III metals;
4. Salts of strongly basic organic nitrogen-containing compounds, including:

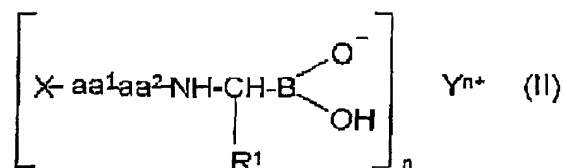
4A. Salts of guanidines and their analogues;

4B. Salts of strongly basic amine, examples of which include (i) aminosugars and (ii) other amines.

Of the above salts, the most preferred are alkali metals, especially Na and Li, and aminosugars.

The preferred salts are of the monovalent boronate (i.e. a single one of the B-OH groups is ionised) though in practice the monovalent salts may contain a very small proportion of the divalent boronate.

The invention includes therefore products (compositions of matter) which comprise salts of formula (II):



where Y^{n+} is a pharmaceutically acceptable cation obtainable from a strong base, and aa^1 , aa^2 , X and R^1 are as defined above.

The salts preferably have a solubility of at least 10 mM, more preferably at least 20mM, when their solubility is determined as described in the examples at a dissolution of 25mg/ml. More preferably yet they have a solubility of least 50mM when their solubility is determined as described in the examples at a dissolution of 50mg/ml.

P41070GB1.2 - 50c Salts/Generic Salts I

11

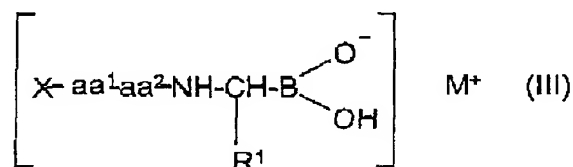
Considering the counter-ions in turn:

1. Alkali metal salts

- 5 Suitable alkali metals include lithium, sodium and potassium. All of these are remarkably soluble. Lithium and sodium are particularly preferred because of their high solubility. The lithium and particularly sodium salts are of surprisingly high solubility in relation to potassium amongst others. Sodium is most preferred. Salts containing mixtures of alkali metals are contemplated by the invention.

10

The invention includes products comprising salts of the formula

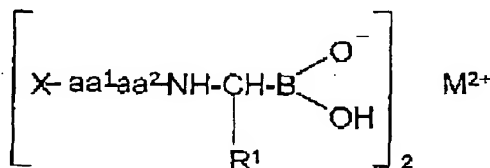


- 15 where M^+ is an alkali metal ion and aa^1 , aa^2 , X and R^1 are as defined above, as well as salts in which both hydroxy groups of the boronate group are in salt form (preferably with another identical M^+ group) and mixtures of such salts.

2. Alkaline earth metal salts

- 20 A suitable alkaline earth metal is calcium. As the alkaline earth metals are divalent, they are usually used in a boronic acid:metal ratio of substantially 2:1, in order to achieve the preferred monovalent boronate moiety. Salts containing mixtures of alkaline earth metals are contemplated by the invention. The alkaline earth metals are indicated to be of no more than moderate solubility and are less preferred.

- 25 The invention includes products comprising salts of the formula:



P41070GB1.2 - 50c Salts/Generic Salts I

12

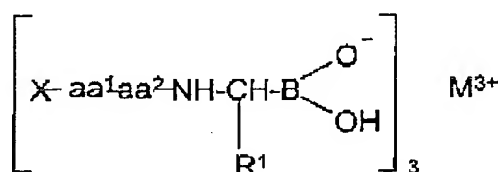
where M^{2+} is an alkaline earth metal ion and aa^1 , aa^2 , X and R^1 are as defined above, as well as salts in which both hydroxy groups of the boronate group are in salt form (preferably with another identical M^{2+} group) and mixtures of such salts.

5 3. Group III metals

Suitable Group III metals include aluminium and gallium. Salts containing mixtures of Group III metals are contemplated by the invention. The Group III metals may be of no more than moderate solubility and are less preferred.

10

The invention includes products comprising salts of the formula:



where M^{3+} is a Group III metal ion and aa^1 , aa^2 , X and R^1 are as defined above, as well as salts in which both hydroxy groups of the boronate group are in salt form (preferably with another identical M^{3+} group) and mixtures of such salts.

15

4. Strongly basic organic nitrogen-containing compounds

The invention includes products obtainable by (having the characteristics of a product obtained by) reaction of a peptide boronic acid as defined above and a strong organic base. Two preferred classes of organic base are described in sections 4A and 4B below. Particularly preferred are acid salts (in which one of the two boronic -OH groups is deprotonated). Most commonly, the salts contain a single type of organic counter-ion (disregarding trace contaminants) but the invention contemplates salts containing mixtures of organic counter-ions; in one sub-class, the different counter-ions all fall within the section 4A family described below or, as the case may be, in the section 4B family below; in another subclass, the salts comprise a mixture of organic counter-ions which are not all from the same family (4A or 4B).

Suitable organic bases include those with a pK_b of 7 or more, e.g. 7.5 or more, for example in the region of 8 or more. Bases which are less lipophilic [e.g. have at least one polar functional group (e.g. 1, 2 or 3 such groups) for example hydroxy] are favoured; thus aminosugars are one favoured class of base.

30

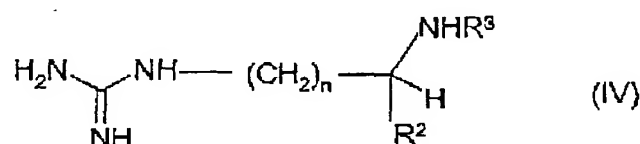
4A. Guanidines and their analogues

The guanidino compound (guanidine) may in principle be any soluble and pharmaceutically acceptable compound having a guanidino or a substituted guanidino group, or a substituted or unsubstituted guanidine analogue. Suitable substituents include aryl (e.g. phenyl), alkyl or alkyl interrupted by an ether or thioether linkage and, in any event, typically contain from 1 to 6 and especially 1, 2, 3, or 4 carbon atoms, as in the case of methyl or ethyl. The guanidino group may have 1, 2, 3 or 4 substituent groups but more usually has 1 or 2 substituent groups, preferably on a terminal nitrogen. One class of preferred guanidine is monoalkylated; another class is dialkylated. As guanidine analogues may be mentioned thioguanidines and 2-amino pyridines. Compounds having unsubstituted guanidino groups, for example guanidine and arginine, form one particularly preferred class.

Salts containing mixtures of guanidines are contemplated by the invention.

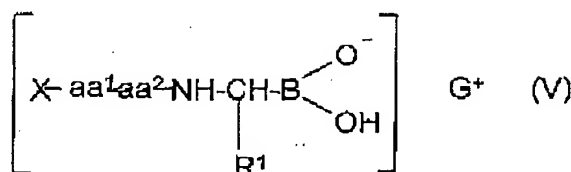
The guanidino compound is preferably L-arginine or an L-arginine analogue, for example D-arginine, or the D- or, preferably, L- isomers of homoarginine or agmatine [(4-aminobutyl) guanidine]. Less preferred arginine analogues are NG-nitro-L-arginine methyl ester, for example, and constrained guanidine analogues, particularly 2-amino pyrimidines, for example 2,6-quinazolinediamines such as 5,6,7,8-tetrahydro-2,6-quinazolinediamine, for example. The guanidino compound may also be a peptide, for example a dipeptide, containing arginine; one such dipeptide is L-tyrosyl-L-arginine.

Some particularly preferred guanidino compounds are compounds of formula (IV):



where n is from 1 to 6 and preferably at least 2, e.g. 3 or more, and preferably no more than 5. Most preferably, n is 3, 4 or 5. R² is H or carboxylate or derivatised carboxylate, for example to form an ester (e.g. a C₁-C₄ alkyl ester) or amide. R³ is H, C₁-C₄ alkyl or a residue of a natural or unnatural amino acid (e.g. tyrosine). The compounds of formula (IV) are usually of L-configuration. The compounds of formula (IV) are arginine (n=3; R²=carboxyl; R³=H) and arginine derivatives or analogues.

The invention includes products comprising salts of the formula



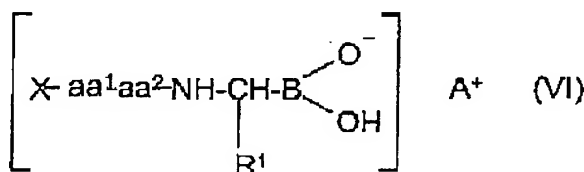
- where aa¹, aa², X and R¹ are as defined previously and G⁺ is the protonated form of a pharmaceutically acceptable organic compound comprising a guanidino group or an analogue thereof, as well as salts in which both hydroxy groups of the boronate group are in salt form (preferably with another identical G⁺ group) and mixtures of such salts.

4B. Strongly basic amines

- The invention includes products obtainable by (having the characteristics of a product obtained by) reaction of a peptide boronic acid as defined above and a strong organic base which is an amine. The amine may in principle be any soluble and pharmaceutically acceptable amine.

- It is envisaged that a desirable class of amine includes those having polar functional groups in addition to a single amine group, as such compounds will be more hydrophilic and thus more soluble than others. Preferably, the or each additional functional group is hydroxy. Some amines have 1, 2, 3, 4, 5 or 6 additional functional groups, especially hydroxy groups. In one particularly preferred class of amines the ratio of (amino plus hydroxy groups):carbon atoms is from 1:2 to 1:1, the latter ratio being particularly preferred. These amines with one or more additional polar functional groups may be a hydrocarbon, especially an alkane, substituted by the amino group and the additional polar group(s). The amino group may be substituted or unsubstituted and, excluding amino substituents, the polar base may contain, for example, up to 10 carbon atoms; usually there are no less than three such carbon atoms, e.g. 4, 5 or 6. Aminosugars are included in this category of polar bases.

- The invention includes products comprising salts of the formula



P41070GB1.2 - 50c Salts/Generic Salts I

15

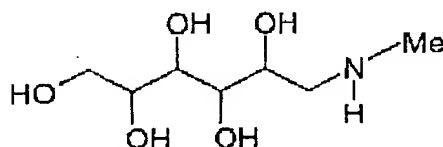
where aa^1 , aa^2 , X and R^1 are as defined previously and A^+ is the protonated form of a pharmaceutically acceptable amine, as well as salts in which both hydroxy groups of the boronate group are in salt form (preferably with another identical A^+ group) and mixtures of such salts. In one class of such products, A^+ is the protonated form of an amine described in section 4B(i) below; in another class A^+ is the protonated form of an amine described in 4B(ii) below

Two preferred classes of amine base are described in sections 4B(i) and 4B(ii) below. Particularly preferred are acid salts (in which one of the two boronic -OH groups is deprotonated). Most commonly, the salts contain a single type of amine counter-ion (disregarding trace contaminants) but the invention contemplates salts containing mixtures of amine counter-ions; in one sub-class, the different counter-ions all fall within the sub-section 4B(i) family described below or, as the case may be, in the sub-section 4B(ii) family below; in another subclass, the salts comprise a mixture of organic counter-ions which are not all from the same family (4B(i) or 4B(ii)).

4B(i) Aminosugars

The identity of the aminosugar is not critical to the invention. Preferred aminosugars include ring-opened sugars, especially glucamines. Cyclic aminosugars are also envisaged as useful. One class of the aminosugars is N-unsubstituted and another, preferred, class is N-substituted by one or two N-substituents (preferably one). Suitable substituents are hydrocarbyl groups, for example and without limitation containing from 1 to 12 carbon atoms; the substituents may comprise alkyl or aryl moieties or both. Preferred substituents are C_1 , C_2 , C_3 , C_4 , C_5 , C_6 , C_7 and C_8 alkyl groups, in particular methyl and ethyl, of which methyl is most preferred. Data indicate that aminosugars, especially N-methyl-D-glucamine, are of surprisingly high solubility.

A most preferred aminosugar is N-methyl-D-glucamine:

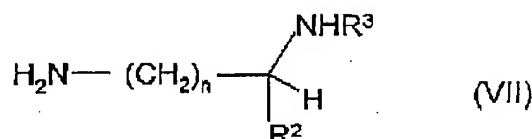


4B(ii) Other amines

Other suitable amines include amino acids (whether naturally occurring or not) whose side chain is substituted by an amino group, especially lysine.

16

Some amines are compounds of formula (VII):



where n , R^2 and R^3 are as defined in relation to formula (IV). The compounds of formula (VI) are usually of L-configuration. The compounds of formula (VI) are lysine ($n=4$; $\text{R}^2=\text{carboxyl}$; $\text{R}^3=\text{H}$) and lysine derivatives or analogues. A most preferred amine is L-lysine.

Other suitable amines are nitrogen-containing heterocycles. At least usually, such heterocyclic compounds are alicyclic; one class of the heterocyclic compounds is N-substituted and another, preferred, class is N-unsubstituted. The heterocycles may contain 6 ring-forming atoms, as in the cases of piperidine, piperazine and morpholine. One class of amines includes N-containing heterocycles substituted by polar substituents, especially hydroxy, e.g. 1, 2 or 3 times.

The invention therefore includes amines other than aminosugars which have one or more (e.g. 1, 2, 3, 4, 5 or 6) polar substituents, especially hydroxy, in addition to one amine group. Such compounds may have a ratio of (amino plus hydroxy groups):carbon atoms of 1:2 to 1:1, the latter ratio being particularly preferred.

The invention includes mixed salts, i.e. salts containing a mixture of boropeptide moieties and/or counterions but single salts are preferred.

The salts in solid form may contain water.

Use of the Products of the Invention

The salts of the invention are potent thrombin inhibitors. They are therefore useful for inhibiting thrombin. The invention therefore provides compounds which have potential for controlling haemostasis and especially for inhibiting coagulation, for example preventing secondary events after myocardial infarction. The medical use of the compounds may be prophylactic (including to prevent occurrence of thrombosis) as well as therapeutic (including to prevent re-occurrence of thrombosis or secondary thrombotic events).

Those salts may be employed when an anti-thrombogenic agent is needed. They are thus indicated in the treatment or prophylaxis of thrombosis and hypercoagulability in blood and tissues of animals including man.

- 5 It is known that hypercoagulability may lead to thrombo-embolic diseases. Conditions associated with hypercoagulability and thrombo-embolic diseases which may be mentioned include activated protein C resistance, such as the factor V-mutation (factor V Leiden), and inherited or acquired deficiencies in antithrombin III, protein C, protein S, heparin cofactor II. Other conditions known to be associated with hypercoagulability and thrombo-embolic disease include circulating
10 antiphospholipid antibodies (Lupus anticoagulant), homocysteinemia, heparin induced thrombocytopenia and defects in fibrinolysis. The thrombin inhibitors of the invention are thus indicated both in the therapeutic and/or prophylactic treatment of these conditions.

- Particular disease states which may be mentioned include the therapeutic and/or prophylactic
15 treatment of venous thrombosis and pulmonary embolism.

- The thrombin inhibitors of the invention are further indicated in the treatment of conditions where there is an undesirable excess of thrombin without signs of hypercoagulability, for example in neurodegenerative diseases such as Alzheimer's disease. In addition to its effects on
20 the coagulation process, thrombin is known to activate a large number of cells (such as neutrophils, fibroblasts, endothelial cells and smooth muscle cells). Therefore, the compounds of the invention may also be useful for the therapeutic and/or prophylactic treatment of idiopathic and adult respiratory distress syndrome, pulmonary fibrosis following treatment with radiation or chemotherapy, septic shock, septicaemia, inflammatory responses, which include, but are not
25 limited to, edema, acute or chronic atherosclerosis such as coronary arterial disease, cerebral arterial disease, peripheral arterial disease, reperfusion damage, and restenosis after percutaneous trans-luminal angioplasty (PTA).

- Moreover, the thrombin inhibitors of the invention are expected to have utility in prophylaxis of
30 re-occlusion (i.e. thrombosis) after thrombolysis, percutaneous trans-luminal angioplasty (PTA) and coronary bypass operations; the prevention of re-thrombosis after microsurgery and vascular surgery in general. Further indications include the therapeutic and/or prophylactic treatment of disseminated intravascular coagulation caused by bacteria, multiple trauma, intoxication or any other mechanism; anticoagulant treatment when blood is in contact with
35 foreign surfaces in the body such as vascular grafts, vascular stents, vascular catheters, mechanical and biological prosthetic valves or any other medical device; and anticoagulant treatment when blood is in contact with medical devices outside the body such as during cardiovascular surgery using a heart-lung machine or in haemodialysis.

The salts of the invention may also be useful in the treatment of pancreatitis.

Administration and Pharmaceutical Formulations

- 5 The compounds may be administered to a host, for example to obtain an anti-thrombogenic effect. In the case of larger animals, such as humans, the compounds may be administered alone or in combination with pharmaceutically acceptable diluents, excipients or carriers. The term "pharmaceutically acceptable" includes acceptability for both human and veterinary purposes, of which acceptability for human pharmaceutical use is preferred. In the case of oral
10 administration, the compounds are preferably administered in a form which prevents the salt of the invention from contact with the acidic gastric juice, such as enterically coated formulations, which thus prevent release of the salt of the invention until it reaches the duodenum.

- The enteric coating is suitably made of carbohydrate polymers or polyvinyl polymers, for
15 example. Examples of enteric coating materials include, but are not limited to, cellulose acetate phthalate, cellulose acetate succinate, cellulose hydrogen phthalate, cellulose acetate trimellitate, ethyl cellulose, hydroxypropyl-methylcellulose phthalate, hydroxypropylmethylcellulose acetate succinate, carboxymethyl ethylcellulose, starch acetate phthalate, amylose acetate phthalate, polyvinyl acetate phthalate, polyvinyl butyrate phthalate, styrene-maleic acid copolymer, methyl-
20 acrylate-methacrylic acid copolymer (MPM-05), methylacrylate-methacrylic acid-methylmethacrylate copolymer (MPM-06), and methylmethacrylate-methacrylic acid co-polymer (Eudragit® L & S). Optionally, the enteric coating contains a plasticiser. Examples of the plasticiser include, but are not limited to, triethyl citrate, triacetin, and diethyl phthalate.

- 25 The salts of the invention may also be combined and/or co-administered with any antithrombotic agent with a different mechanism of action, such as the antiplatelet agents acetylsalicylic acid, ticlopidine, clopidogrel, thromboxane receptor and/or synthetase inhibitors, fibrinogen receptor antagonists, prostacyclin mimetics and phosphodiesterase inhibitors and ADP-receptor (P_2 T) antagonists.

- 30 The salts of the invention may further be combined and/or co-administered with thrombolytics such as tissue plasminogen activator (natural, recombinant or modified), streptokinase, urokinase, prourokinase, anisoylated plasminogen-streptokinase activator complex (APSAC), animal salivary gland plasminogen activators, and the like, in the treatment of thrombotic
35 diseases, in particular myocardial infarction.

Typically, therefore, the products of the invention may be administered to a host to obtain a thrombin-inhibitory effect.

Actual dosage levels of active ingredients in the pharmaceutical compositions of this invention may be varied so as to obtain an amount of the active compound(s) that is effective to achieve the desired therapeutic response for a particular patient, compositions, and mode of administration. The selected dosage level will depend upon the activity of the particular compound, the severity of the condition being treated and the condition and prior medical history of the patient being treated. However, it is within the skill of the art to start doses of the compound at levels lower than required for to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

According to a further aspect of the invention there is thus provided an oral pharmaceutical formulation including a product of the invention, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

Solid dosage forms for oral administration include capsules, tablets, pills, powders and granules. In such solid dosage forms, the active compound is typically mixed with at least one inert, pharmaceutically acceptable excipient or carrier such as sodium citrate or dicalcium phosphate and/or one or more: a) fillers or extenders such as starches, lactose, sucrose, glucose, mannitol and silicic acid; b) binders such as carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidone, sucrose and acacia; c) humectants such as glycerol; d) disintegrating agents such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates and sodium carbonate; e) solution retarding agents such as paraffin; f) absorption accelerators such as quaternary ammonium compounds; g) wetting agents such as cetyl alcohol and glycerol monostearate; h) absorbents such as kaolin and bentonite clay and i) lubricants such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycol, for example.

Suitably, the oral formulations may contain a dissolution aid. The dissolution aid is not limited as to its identity so long as it is pharmaceutically acceptable. Examples include nonionic surface active agents, such as sucrose fatty acid esters, glycerol fatty acid esters, sorbitan fatty acid esters (e.g., sorbitan trioleate), polyethylene glycol, polyoxyethylene hydrogenated castor oil, polyoxyethylene sorbitan fatty acid esters, polyoxyethylene alkyl ethers, methoxypolyoxyethylene alkyl ethers, polyoxyethylene alkylphenyl ethers, polyethylene glycol fatty acid esters, polyoxyethylene alkylamines, polyoxyethylene alkyl thioethers, polyoxyethylene polyoxypropylene copolymers, polyoxyethylene glycerol fatty acid esters, pentaerythritol fatty acid esters, propylene glycol monofatty acid esters, polyoxyethylene propylene glycol monofatty acid esters, polyoxyethylene sorbitol fatty acid esters, fatty acid alkylolamides, and alkylamine

P41070GB1.2 - 50c Salts/Generic Salts I

20

oxides; bile acid and salts thereof (e.g., chenodeoxycholic acid, cholic acid, deoxycholic acid, dehydrocholic acid and salts thereof, and glycine or taurine conjugate thereof); ionic surface active agents, such as sodium laurylsulfate, fatty acid soaps, alkylsulfonates, alkylphosphates, ether phosphates, fatty acid salts of basic amino acids; triethanolamine soap, and alkyl quaternary ammonium salts; and amphoteric surface active agents, such as betaines and aminocarboxylic acid salts.

The active compounds may also be in micro-encapsulated form, if appropriate, with one or more of the above-mentioned excipients.

10

Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, solutions, suspensions, syrups and elixirs. In addition to the active compounds, the liquid dosage forms may contain inert diluents commonly used in the art such as water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethyl formamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan and mixtures thereof. Besides inert diluents, the oral compositions may also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavouring and perfuming agents. Suspensions, in addition to the active compounds, may contain suspending agents such as ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminium metahydroxide, bentonite, agar-agar, and tragacanth and mixtures thereof.

The product of the invention may be presented as solids in finely divided solid form, for example they may be micronised.

The active compound may be given as a single dose, in multiple doses or as a sustained release formulation.

30

Synthesis

1. Peptide Synthesis

The synthesis of boropeptides, including, for example, Cbz-D-Phe-Pro-BoroMpg-OPinacol is familiar to those skilled in the art and described in the prior art mentioned above, including Claeson et al (US 5574014 and others) and Kakkar et al (WO 92/07869 and family members including US 5648338). It is described also by Elgendy et al *Adv. Exp. Med. Biol. (USA)* **1993**,

P41070GB1.2 - 50c Salts/Generic Salts I

21

340, 173-178; Claeson, G. et al *Biochem. J.* **1993**, *290*, 309-312; Deadman et al *J. Enzyme Inhibition* **1995**, *9*, 29-41, and by Deadman et al *J. Med. Chem.* **1995**, *38*, 1511-1522.

5 Stereoselective synthesis with S or R configuration at the chiral B-terminal carbon may be conducted using established methodology (Elgendy et al *Tetrahedron. Lett.* **1992**, *33*, 4209-4212; WO 92/07869 and family members including US 5648338) using (+) or (—)- pinanediol as the chiral director (Matteson et al *J. Am. Chem. Soc.* **1986**, *108*, 810-819; Matteson et al *Organometallics.* **1984**, *3*, 1284-1288). Another approach is to resolve the requisite aminoboronate intermediate (e.g. Mpg-BOPinacol) to selectively obtain the desired (R)-isomer
10 and couple it to the dipeptide moiety (e.g. Cbz-(R)-Phe-(S)-Pro, which is the same as Cbz-D-Phe-L-Pro) which will form the remainder of the molecule.

The boropeptides may be synthesised initially in the form of boronic acid esters, particularly esters with diols. Such diol esters may be converted to the peptide boronic acid as described
15 next.

2. Ester to Acid Conversion

A peptide boronate ester such as Cbz-D-Phe-Pro-BoroMpg-OPinacol may be hydrolysed to form
20 the corresponding acid, for example as described in Example 1 below, Section H.

A novel technique for converting a diol ester of a peptide boronic acid of formula (I) into the acid comprises dissolving the diol ester in an ether and particularly a dialkyl ether, reacting the thus-dissolved diol with a diolamine, for example a dialkanolamine, to form a product precipitate,
25 recovering the precipitate, dissolving it in a polar organic solvent and reacting the thus-dissolved product with an aqueous acid to form the peptide boronic acid. The boronic acid may be recovered from the organic layer of the mixture resulting from the reaction, for example by removing the solvent, e.g. by evaporation under vacuum or distillation. The reaction between the diol ester and the diolamine may be carried out under reflux, for example.

30 The identity of the diol is not critical to the invention. As suitable diols may be mentioned aliphatic and aromatic compounds having hydroxy groups that are substituted on adjacent carbon atoms or on carbon atoms substituted by another carbon. That is to say, suitable diols include compounds having at least two hydroxy groups separated by at least two connecting
35 carbon atoms in a chain or ring. A particularly preferred diol is pinacol and other exemplary diols include pinanediol (also a preferred diol), neopentylglycol, diethanolamine, 1,2-ethanediol, 1,2-propanediol, 1,3-propanediol, 2,3-butanediol, 1,2-diisopropylethanediol, 5,6-decanediol and 1,2-dicyclohexylethanediol.

P41070GB1.2 - 50c Salts/Generic Salts I

22

The alkyl groups of the dialkyl ether preferably have 1, 2, 3 or 4 carbon atoms and the alkyl groups may be the same or different. A most preferred ether is diethyl ether.

5 The alkyl groups of the dialkanolamine preferably have 1, 2, 3 or 4 carbon atoms and the alkyl groups may be the same or different. A most preferred dialkanolamine is diethanolamine.

The polar organic solvent is preferably CHCl_3 .

10 The aqueous acid is suitably a strong inorganic acid at a pH in the region of 1; hydrochloric acid is most preferred.

After reaction with the acid, the reaction mixture is suitably washed with, for example, NH_4Cl .

A preferred procedure is as follows

- 15 1. The pinacol ester of the selected peptide boronic acid is dissolved in diethylether.
2. Diethanolamine is added and the mixture is refluxed at 40 °C.
3. The precipitated product is removed, washed (usually several times) with diethylether and dried (e.g. by evaporation under vacuum).
4. The dry product is dissolved in CHCl_3 . Hydrochloric acid (pH 1) is added and the mixture is
20 stirred approximately 1h at room temperature.
5. The organic layer is removed and washed with NH_4Cl solution.
6. The organic solvent is distilled off and the residual solid product is dried.

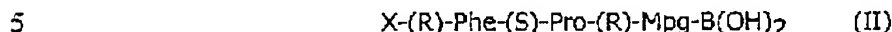
25 The above process results in the formation of an ester-amide of the peptide boronic acids of formula (I), especially ester-amides with diethanolamine, and such ester-amides are themselves included in the invention.

30 The invention provides also the use of a peptide boronic acid of formula (I) to make a salt of the invention. Included also is a method of preparing a product of the invention, comprising contacting a peptide boronic acid of formula (I) with a base capable of making such a salt.

The peptide boronic acid of formula (I) used to prepare the pharmaceutical preparations is typically of GLP or GMP quality, or in compliance with GLP (good laboratory practice) or GMP (good manufacturing practice); such acids are included in the invention.

35 Similarly the acids are usually sterile and/or acceptable for pharmaceutical use, and one aspect of the invention reside in a composition of matter which is sterile or acceptable for pharmaceutical use, or both, and comprises a peptide boronic acid of formula (I). Such a composition of matter may be in particulate form or in the form of a liquid solution or dispersion.

The intermediate acid may be in isolated form and such isolated acids are included in the invention, especially isolated acids which are a peptide boronic acid of formula (II):



wherein X is H (to form NH₂) or an amino-protecting group.

One typical way of providing the intermediate acids is as a particulate composition consisting predominantly of such a peptide boronic acid, and these compositions are included in the invention. The peptide boronic acid often forms at least 75% by weight of the composition and typically at least 85% by weight of the composition, e.g. at least 95% by weight of the composition.

15 Another typical way of providing the intermediate acids is as a liquid composition consisting of, or consisting essentially of, a peptide boronic acid of formula (II) and a liquid vehicle in which it is dissolved or suspended. The liquid vehicle may be an aqueous medium, e.g. water, or an alcohol, for example methanol, ethanol, isopropanol, or another propanol, another alkanol or a mixture of the foregoing.

20

The compositions of the intermediate acids are generally sterile. The compositions may contain the peptide boronic acid in finely divided form, to facilitate further processing.

3. Salt Synthesis

25

The salts may be prepared by contacting the relevant peptide boronic acid with a strong base appropriate to form the desired salt. In the case of metal salts, the metal hydroxides are suitable bases (alternatively, metal carbonates might be used, for example), whilst salts with organic bases may be prepared by contacting the peptide boronic acid with the organic base itself. The preferred salts of the invention are acid salts (one -BOH proton replaced) and, to make these salts, the acid and the base, if monovalent, are usually reacted in substantially equimolar quantities; where calcium or another divalent cation is used, the usual acid:base molar ratio is substantially 2:1, whilst the usual acid: base molar ratio is substantially 3:1 for aluminium or another trivalent cation. Generally stated therefore, the usual acid base molar ratio is substantially n:1, where n is the valency of the cation of the base.

Typically, a solution of the peptide boronic acid in a water-miscible organic solvent, for example acetonitrile or an alcohol (e.g. ethanol, methanol, a propanol, especially iso-propanol, or another

P41070GB1.2 - 50c Salts/Generic Salts I

24

alkanol), is combined with an aqueous solution of the base. The acid and the base are allowed to react and the salt is recovered. The reaction is typically carried out at ambient temperature (e.g. at a temperature of from 15 to 25°C), but an elevated temperature may be used, for example up to the boiling point of the reaction mixture but more usually lower, e.g. a temperature of up to 40°C or 50°C. The reaction mixture may be allowed to stand or be agitated (usually stirred).

The time during which the acid and the base are allowed to react is not critical but it has been found desirable to maintain the reaction mixture for at least one hour. A period of from one to two hours is usually suitable but longer reaction times are included in the invention.

The salt may be recovered from the reaction mixture by any suitable method, for example evaporation, precipitation or crystallisation. In one preferred technique, the salt is recovered by evacuating the reaction mixture to dryness. The salt is preferably thereafter purified, for example by redissolving the salt before filtering the resulting solution and drying it, for example by evacuating it to dryness or freeze drying. The redissolution may be performed using water, e.g. distilled water. The salt may then be further purified, for example in order to remove residual water by further redissolution in a suitable solvent, which is advantageously ethyl acetate followed by evaporating to dryness. The purification procedure may be carried out at ambient temperature (say, 15 to 25°C), or at a modestly elevated temperature, such as e.g. a temperature not exceeding 40°C or 50°C; for example the salt may be dissolved in water and/or solvent by agitating with or without warming to, for example, 37°C.

The invention includes a method for drying the salts of the invention and other peptide boronic acid salts, comprising dissolving them in ethyl acetate and then evaporating to dryness, e.g. by evacuation.

A preferred general procedure for synthesising salts of Cbz-Phe-Pro-BoroMpg-OH is as follows:

Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added the requisite base as a 0.2M solution (0.1M for a divalent cation; 0.67M for a trivalent cation) in distilled water (190ml). The resultant clear solution is allowed to react for example by being left to stand or being agitated, for a usual period, in either case, of from one to two hours. The reaction is typically carried out at ambient temperature (e.g. 15-25°C) but alternatively the temperature may be elevated (e.g. up to 30°C, 40°C or 50°C). The reaction mixture is then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant oil/tacky liquid is redissolved in the minimum amount of distilled water necessary (200ml to 4L), typically with warming (e.g. to 30-40°C), usually for up to 2 hours. The solution is filtered, suitably through filter paper, and evacuated to

P41070GB1.2 - 50c Salts/Generic Salts I

25

dryness, again with the temperature of the solution not exceeding 37°C, or freeze dried. The resultant product is dried under vacuum overnight to normally yield a white brittle solid. If the product is present as an oil or tacky solid then it is dissolved in ethyl acetate and evacuated to dryness to produce the product as a white solid.

5

In variations of the foregoing general procedure, the acetonitrile is replaced by another water-miscible organic solvent, notably an alcohol, as discussed above, especially ethanol, methanol, iso-propanol or another propanol.

10 ***Separation of Stereoisomers***

The stereoisomers of a peptide boronic acid or a synthetic intermediate aminoboronate may be resolved in, for example, any known way. Accordingly, they may be resolved by chromatography (HPLC) or salt crystallisation.

15

Examples

The following compounds are referred to in the Examples:

20 TRI50b = Cbz-Phe-Pro-BoroMpg-OPinacol.

TRI50c = Cbz-Phe-Pro-BoroMpg-OH. This is the free acid of TRI50b.

It is considered that the TRI50b and TRI50c featured in the examples are at least predominantly of the most active isomer, considered to be of RSR (DLL) configuration, as discussed above.

25

The solubility data presented in the examples were obtained from salts made using a modification of the salt preparation process described in the examples. The modified process differs from that described in the examples in that 100mg of TRI50c was used as starting material, the product of the redissolution in water was dried by freeze drying and the filtration was carried out through a 0.2µm filter. The salts for which solubility data are presented are believed to contain about 85% of the most active isomer, considered to be of RSR configuration.

30

When repeated with very pure active isomer salts obtained using the procedure described in the example from isomerically pure TRI50c, the solubility data were the same as those presented within experimental error or very slightly higher.

35

EXAMPLE 1 – SYNTHESIS OF TRI50C

A. 3-METHOXYPROPENE

P41070GB1.2 - 50c Salts/Generic Salts I

26

1. REAGENTS AND CONSUMABLES**1.1 SPECIFICATIONS**

5 1,4-Dioxan (SPS).

Toluene, AR grade.

Allyl Alcohol.

10

Sodium Hydride as 60% dispersion in mineral oil. It should be a pale grey powder. Overall white colour indicates decomposition.

Dimethyl sulphate.

15

Magnesium sulphate dried (SLR).

Water, standard laboratory purified water is used throughout.

20 Nitrogen, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

Argon, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

25

1.2 PURIFICATION OF REAGENTS

All glassware used in these purification steps is heated at 140-160°C for at least 4 hours and then cooled in a desiccator or by assembling while hot and purging with a stream of dry nitrogen before use.

30

2 APPARATUS

Standard laboratory glassware and specialised apparatus for handling and transferring of air sensitive reagents is used for this preparation procedure.

35 All glassware is heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen or argon.

P41070GB1.2 - 50c Salts/Generic Salts I

27

The mechanical stirrer should be of sufficient torque to stir a viscous suspension. The stirrer arm should be fitted to the flask through a quickfit sleeve with inert oil seal.

Reaction is conducted in a three necked flask, to allow overhead stirring, inert gas purge and sodium hydride addition. A heating mantle of appropriate size is required.

3 PROCEDURE

3.1 PREPARATION

To a mechanically stirred cooled solution under nitrogen with a gas outlet and fitted with a water condenser of allyl alcohol (107.8ml, 1.59mol) and dimethylsulphate (200ml, 1.59mol, 1.eq.) in 1,4-dioxane (1L) is added, portionwise NaH (60% dispersion in mineral oil, 63.5g, 1.59mol, 1eq.). Care is taken that the reaction temperature remains at or below room temperature and the reaction is stirred until effervescence has ceased.

3.2 PURIFICATION AND WORK-UP

The slurry is stirred, carefully, into ice (1L), and extracted with toluene (3x500ml). The organic phase is heated (mantle) with a fractionation column, to distil off at atmospheric pressure the methoxypropene, b.p. 45-60°C. Heating should be observed to keep the vapour temperature in the 45-60°C range, since unreacted allyl alcohol distils at 96-98°C.

3.3 CHARACTERISATION AND CONFIRMATION OF PRODUCT

The distilled 3-methoxypropene should be checked by ^1H NMR spectroscopy.

B. 3-METHOXYPROPYL BORONATE CATECHOL ESTER

1 REAGENTS AND CONSUMABLES

1.1 SPECIFICATIONS

Catecholborane. The appearance should be a low melting (m.p. 12°C) solid.

3-methoxypropene. The appearance should be a clear volatile liquid. It must be stored at below 4°C.

Nitrogen, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

Argon, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

P41070GB1.2 - 50c Salts/Generic Salts I

28

2 APPARATUS

Standard laboratory glassware and specialised apparatus for handling and transferring of air sensitive reagents is used for this preparation procedure.

A heat gun or water bath is required to prewarm the bottle of catecholborane.

All glassware must be heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen or argon.

3 PROCEDURE

3.1 PREPARATION

15

To 3-methoxypropene (120g, 1.66mol) in a 1l flask cooled in an ice bath and fitted with a condenser, is added, dropwise by dry transfer via a dropping funnel, catecholborane (199.6g, 1eq.) (which is prewarmed, if necessary, to give a liquid) and left overnight at room temperature. Careful addition of the catecholborane is necessary as the reaction can become violently exothermic.

20

The mixture is heated at 60-70°C for 24hrs. The mixture is allowed to cool to room temperature.

3.2 PURIFICATION AND WORK-UP

There is no purification at this stage. Used immediately.

25

3.3 CHARACTERISATION AND CONFIRMATION OF PRODUCT

The catechol 3-methoxypropyl boronate should be checked by ^1H NMR spectroscopy. ^{13}C Signals should be observed as follows:-

δ_{60}	Signal Pattern	Assignment
7.13	4H, multiplet	Ph
3.4	2H, multiplet	CH_2OMe
3.39	3H, singlet	OMe
1.92	2H, multiplet	CH_2
1.29	2H, multiplet	CH_2

30

Observation of other signals would be indicative of impurities

P41070GB1.2 - 50c Salts/Generic Salts I

29

C. 3-METHOXYPROPYL BORONATE PINACOL ESTER**1 REAGENTS AND CONSUMABLES****1.1 SPECIFICATIONS**

Pinacol.

10 Nitrogen, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

Argon, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

2 APPARATUS

Standard laboratory glassware and specialised apparatus for handling and transferring of air sensitive reagents is used for this preparation procedure.

20 All glassware must be heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen or argon.

25 3 PROCEDURE**3.1 PREPARATION**

To catechol 3-methoxypropaneboronate (1.66mol, from section B2) is added, at 0°C, pinacol (126g, 1eq). The solution is stirred at 0°C for 1hr. Remove the ice bath and leave at room temperature overnight.

3.2 PURIFICATION AND WORK-UP

35 To a 3l flask containing 1.5l hexane (lab. grade, not dried) transfer the solution from 3.1. Allow the catechol to precipitate out (storage at <40°C for 1-2 hrs. facilitates this) and decant off the hexane into a 3l separating funnel. Wash the precipitate with a further 500ml of hexane and add to the first hexane solution. Wash the hexane with water (2x500ml, analytical grade). Back extract each aqueous wash with (2x500ml) hexane. Dry the hexane layer with anhydrous MgSO₄. Filter (glass sinter, grade four).

P41070GB1.2 - 50c Salts/Generic Salts I

30

Remove the solvent using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

5 3.3 CHARACTERISATION AND CONFIRMATION OF PRODUCT

The pinacol 3-methoxypropyl boronate product should be checked by ^1H NMR spectroscopy $^\circ\text{C}$. Signals should be observed as follows:-

δ_{400}	δ_{60}	Signal Pattern	Assignment
3.33-3.37	-	5H, multiplet	$\text{CH}_2\text{-O-CH}_3$
1.69	-	2H, multiplet	CH_2CH_2
1.24	-	12H, singlet	pinacol
0.79	-	2H, multiplet	CH_2B

10 Due to the presence of impurities other signals will be observed also.

If impurity levels are unacceptable, distil the product (bp. $55^\circ\text{C}/0.4\text{mmHg}$, pinacol 3-methoxypropyl boronate).

15 D. 4-METHOXY-1-CHLOROBUTYL BORONATE PINACOL ESTER

1 REAGENTS AND CONSUMABLES

1.1 SPECIFICATIONS

20

Dichloromethane (AR) dried/redistilled before use.

Tetrahydrofuran (AR) dried/redistilled before use.

25 Hexane (AR).

Lithium diisopropylamide, 2.0M in hexane/tetrahydrofuran/ethylbenzene. The reagent must be inspected before each use. It should be a clear pale red/brown solution. If it deviates from this colour or has any white precipitate it must be discarded. Store at $<6^\circ\text{C}$.

30

Zinc chloride, 0.5M in THF.

Cyclohexane, anhydrous, 99.5%.

P41070GB1.2 - 50c Salts/Generic Salts I

31

Benzophenone (SLR).

Sodium metal stored under paraffin oil (SLR).

5

Phosphorus pentoxide (SLR).

Magnesium sulphate dried (SLR).

10 Water, Ultra Pure grade.

Carbon tetrachloride (GLR)

Dry ice.

15

Nitrogen, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

20 Argon, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

1.2 PURIFICATION OF REAGENTS

25 All glassware used in these purification steps is heated at 140-160°C for at least 4 hours and then cooled in a desiccator or by assembling while hot and purging with a stream of argon before use.

1.2.1 Dichloromethane

30 Add phosphorus pentoxide to the dichloromethane at the rate of ca. 10 g per 100cm³ and leave to stand in a stoppered flask for at least 30 minutes. Distil the dichloromethane from the phosphorus pentoxide under a stream of dry nitrogen. The purified solvent is used immediately

1.2.2 Tetrahydrofuran

35 The distillation apparatus is normally set up in the laboratory ready for use and will contain tetrahydrofuran over sodium containing benzophenone (ca. 0.5 g per litre) as an indicator. If necessary top up the distillation flask with more tetrahydrofuran so that it is at most two thirds full. If the colour of the solvent in the distillation flask is not blue add sodium (in oil) in small pieces, ca. 5 mm cubes until a blue colour develops. Distil the solvent from the sodium under a stream of dry nitrogen.

The purified tetrahydrofuran is used immediately and stored.

2 APPARATUS

Standard laboratory glassware and specialised apparatus for handling and transferring of air sensitive reagents are used for this preparation procedure.

All glassware is heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen or argon.

3 PROCEDURE

3.1 PREPARATION

To a solution (0.4M, in a 10l flask) of pinacol 3-methoxypropylboronate ester (150g, 0.750mol) in anhydrous cyclohexane (1250ml) and THF (625ml) (section 1.2.2) cooled to -20°C in a carbon tetrachloride/dry ice bath, is added dry DCM (section 1.2.1, 1.22eq., 58.5ml, 0.915mol). Added to this solution (with stirring, under stream of dry argon) dropwise, to maintain the temperature between -20 °C and -15 °C, is lithium diisopropylamide (1.11eq., 416ml, 0.833mol, diluted in 500ml THF) and then zinc chloride (0.5M solution in THF, 1500ml) pre cooled in ice. The reaction is allowed to warm to room temperature overnight.

3.2 PURIFICATION AND WORK-UP

The reaction mixture is diluted in hexane (2l) and poured into cold 1M sulphuric acid (1l), stir for 15 mins, and then extract with hexane (2x500ml). Wash the combined extracts with saturated NaHCO₃ solution (1l), saturated NaCl solution (1l). Dry the combined hexane extracts with anhydrous MgSO₄.

Filter immediately with a grade four glass sinter.

Remove the solvent using a rotary evaporator at room temperature and with a vacuum of ca. 1 mm/Hg. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

P41070GB1.2 - 50c Salts/Generic Salts I

33

3.3 CHARACTERISATION AND CONFIRMATION OF PRODUCT

The unpurified pinacol 4-methoxy-1-chlorobutylboronate should be checked by ^1H NMR spectroscopy. Signals should be observed as follows:-

δ_{400}	Signal Pattern	Assignment
3.47-3.38	3H, multiplet	CH_2OMe and CHB
3.34	3H, singlet	OMe
2.0-1.62	4H, multiplet	CH_2CH_2
1.27	12H, singlet	pinacol

Due to the presence of impurities other signals will be observed also.

E. 4-METHOXY-1-BIS (TRIMETHYLSILYL) AMINO BUTYL BORONATE PINACOL ESTER**1 REAGENTS AND CONSUMABLES****1.1 SPECIFICATIONS**

Tetrahydrofuran (AR) dried/redistilled before use.

n-Hexane SPS grade dried/redistilled before use.

Lithium bis(trimethylsilyl)amide, 1N solution in anhydrous hexane.

Water, Ultra Pure grade.

Nitrogen, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

Argon, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

1.2 PURIFICATION OF REAGENTS

All glassware used in these purification steps is heated at 140-160°C for at least 4 hours and then cooled in a desiccator or by assembling while hot and purging with a stream of dry nitrogen before use.

P41070GB1.2 - 50c Salts/Generic Salts I

34

1.2.1 Tetrahydrofuran

The distillation apparatus is normally set up in the laboratory ready for use and will contain tetrahydrofuran over sodium containing benzophenone (ca. 0.5 g per litre) as an indicator. If necessary top up the distillation flask with more tetrahydrofuran so that it is at least two thirds full. If the colour of the solvent in the distillation flask is not blue add sodium in oil in small pieces, ca. 5 mm cubes, until a blue colour develops. Distil the solvent from the sodium under a stream of dry nitrogen.

The purified tetrahydrofuran is used immediately and not stored.

2 APPARATUS

Standard laboratory glassware and specialised apparatus for handling and transferring of air sensitive reagents is used for this preparation procedure. All glassware must be heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen or argon.

3 PROCEDURE

3.1 PREPARATION

A 0.33M solution of pinacol 4-methoxy-1-chlorobutaneboronate (150g, 0.60mol) in THF (1810ml) is added to a 0.5M solution of lithium hexamethyldisilazane (1N in hexane, 604ml, 1eq) in THF (603ml) at -78°C (dry ice/acetone bath) giving a final concentration of boronate at 0.2M. The reaction mixture is allowed to warm slowly to room temperature and is stirred for at least 12hrs.

3.2 PURIFICATION AND WORK-UP

Remove the solvent using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

Hexane (lab grade, 1000ml) is added to yield a precipitate which is removed by washing with water (2x750ml, analytical grade). Back extract each aqueous phase with (500ml) hexane. Dry the hexane layer with anhydrous MgSO_4 and filter through a grade 4 glass sinter. The organic phase is concentrated using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

The residual oil is distilled under reduced pressure to give b.p. 80-104°C, 0.1 - 0.2 mmHg pinacol 4-methoxy-1-bis(trimethylsilyl)aminobutyl boronate.

P41070GB1.2 - 50c Salts/Generic Salts I

35

3.3 CHARACTERISATION AND CONFIRMATION OF PRODUCT

The distilled pinacol 4-methoxy-1-bis(trimethylsilyl)aminobutyl boronate should be checked by

^1H NMR spectroscopy $^\circ\text{C}$. Signals should be observed as follows:-

δ_{400}	Signal Pattern	Assignment
3.23-3.26	5H, multiplet	$\text{CH}_2\text{O CH}_3$
2.41	1H, multiplet	$\text{CH}_2 \text{CHB}$
1.62	2H, multiplet	$\text{CH}_2 \text{CH}_2$
1.46	1H, multiplet	1H from CH_2 (split H)
1.31	1H, multiplet	1H from CH_2 (split H)
1.12	12H, singlet	pinacol

5

Due to the presence of impurities other signals will be observed also.

F. 4-METHOXY-1-AMINOBTYL BORONATE PINACOL ESTER**10 1. REAGENTS AND CONSUMABLES****1.1 SPECIFICATIONS**

n-Hexane SPS grade dried/redistilled before use.

15

Chloroform (AR) dried/redistilled before use.

HCL, 4N anhydrous solution in 1,4-dioxan.

20 Nitrogen, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

Argon, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

25

1.2 PURIFICATION OF REAGENTS

All glassware used in these purification steps is heated at $140-160^\circ\text{C}$ for at least 4 hours and then cooled in a desiccator or by assembling while hot and purging with a stream of dry nitrogen before use.

30

P41070GB1.2 - 50c Salts/Generic Salts I

36

1.2.1 n-Hexane

Add calcium hydride to the n-hexane at the rate of ca. 10 g per 100cm³ and leave to stand in a stoppered flask for at least 30 minutes. Distil the hexane from the calcium hydride under a stream of dry nitrogen. The purified solvent should be used immediately wherever possible but
5 may be stored for up to 24 hours in a tightly stoppered flask.

1.2.2 Chloroform.

Add phosphorus pentoxide to the chloroform at the rate of ca. 10 g per 100cm³ and leave to stand in a stoppered flask for at least 30 minutes. Distil the chloroform from the phosphorus
10 pentoxide under a stream of dry nitrogen. The purified solvent should be used immediately wherever possible but may be stored for up to 24 hours in a tightly stoppered flask.

2 APPARATUS

Standard laboratory glassware and specialised apparatus for handling and transferring of air
15 sensitive reagents is used for this preparation procedure

All glassware is heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen or argon.

20 3 PROCEDURE**3.1 PREPARATION**

To a 0.4M solution of pinacol 4-methoxy-1-bis(trimethylsilyl)aminobutane boronate (160g, 0.428mol) in dry hexane (1072ml, section 1.2.1) at -78°C (dry ice/acetone), is added HCl(4N,
25 solution in dioxane, 322ml, 3eq.) from a measuring cylinder. The reaction is allowed to warm to room temperature overnight.

3.2 PURIFICATION AND WORK-UP

Remove the solvent using a rotary evaporator under oil pump vacuum. The rotating flask should
30 be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

Dry chloroform (2l, section 1.2.2) is added. The solution is then filtered through celite under nitrogen pressure in a closed system (grade four glass sinter). Organic phase is concentrated
35 using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

P41070G81.2 - 50c Salts/Generic Salts I

37

3.3 CHARACTERISATION AND CONFIRMATION OF PRODUCT

Pinacol 4-methoxy-1-aminobutyl boronate should be checked by electrospray mass spectrometry.

The signals observed should be:

Signal (AMU)	Assignment
253	[M+Na]
230	[M+]

5

Due to the presence of impurities other signals will be observed also.

G. Cbz-D-Phe-Pro-BoroMpg-OPinac (TRI50b)**10 1. REAGENTS AND CONSUMABLES****1.1 SPECIFICATIONS**

Tetrahydrofuran (AR) dried/redistilled before use.

15

n-Hexane SPS grade.

Isobutylchloroformate.

20 N-methylmorpholine.

Triethylamine.

Benzophenone (SLR).

25

Sodium Chloride (SLR).

Sodium bicarbonate (SLR).

30 Hydrochloric Acid (SLR).

Sodium metal stored under paraffin oil (SLR).

Magnesium sulphate dried (SLR).

35

P41070GB1.2 - 50c Salts/Generic Salts I

38

Water, Ultra Pure grade.

Nitrogen, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

5

Argon, laboratory oxygen free grade which is passed through a drying tube packed with self indicating silica gel when required to be dry.

1.2 PURIFICATION OF REAGENTS

- 10 All glassware used in these purification steps is heated at 140-160°C for at least 4 hours and then cooled in a desiccator or by assembling while hot and purging with a stream of dry nitrogen before use.

1.2.1 Tetrahydrofuran

- 15 The distillation apparatus is normally set up in the laboratory ready for use and will contain tetrahydrofuran over sodium containing benzophenone (ca. 0.5 g per litre) as an indicator. If necessary top up the distillation flask with more tetrahydrofuran so that it is at least two thirds full. If the colour of the solvent in the distillation flask is not blue add sodium in oil in small pieces, ca. 5 mm cubes, until a blue colour develops. Distil the solvent from the sodium under a
20 stream of dry nitrogen.

The purified tetrahydrofuran is used immediately and not stored.

2 APPARATUS

- 25 Standard laboratory glassware and specialised apparatus for handling and transferring of air sensitive reagents is used for this preparation procedure.

All glassware is heated at 140-160°C for at least 4 hours before use and then cooled either in a desiccator or by assembling hot and purging with a stream of dry nitrogen or argon.

30

3 PROCEDURE

3.1 PREPARATION

- To a 0.5M solution of Cbz-D-Phe-Pro (0.515mol, 204.5g, 1eq) in THF (1042ml) is added N-methylmorpholine (56.8ml, 1eq.) and the solution cooled to -20°C (CCl₄/dry ice bath). ^tBuOCOCl (67ml, 1eq, in 149ml THF, 3.5M) is added making sure the temperature stays in the range of -20
35 0°C to -15°C. After 15 mins, to the mixture, is added by dry transfer a 1.36M solution of pinacol 4-methoxy-1-aminobutylboronate hydrochloride (150g, 0.57mol, 1.05eq) as a precooled solution

P41070GB1.2 - 50c Salts/Generic Salts I

39

in CHCl_3 (416ml), then Et_3N (75.3ml, 1.05eq) is added. The reaction is allowed to warm to room temperature and stirred for at least 2hrs.

3.2 PURIFICATION AND WORK-UP

- 5 Remove the solvent using a rotary evaporator under oil pump vacuum. The rotating flask should be surrounded by a water bath at room temperature. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

- 10 The residue is dissolved in ethyl acetate (1500ml) and washed with HCl (0.2M, 2x500ml), back extract the combined HCl washes with ethyl acetate (500ml) and combine with ethyl acetate layer. Wash combined ethyl acetate with water (1000ml), back extract the water wash with 500ml of ethyl acetate combined with ethyl acetate layer, NaHCO_3 (saturated aqueous, 2x1000ml) and NaCl (saturated aqueous, 500ml). To the organic phase is added dried magnesium sulphate until it flocculates, the flask stoppered tightly and left to stand for at least
- 15 30 minutes. Remove the magnesium sulphate by filtration through a glass sinter, (grade four). Remove the solvent using a rotary evaporator at room temperature and with a vacuum of ca. 1 mm/Hg. The vacuum and temperature need not be critically determined so long as they are adequate to remove the solvent.

- 20 Leave overnight on high vacuum.

The desired crude product as a foamy solid.

3.3 CHARACTERISATION AND CONFIRMATION OF PRODUCT

25

3.3.1 NMR Analysis

The TRI50b should be checked by ^1H NMR spectroscopy. Signals should be observed as follows:-

δ_{400}	Signal Pattern	Assignment
7.82	1H, broad	NH
7.40-7.20	10H, multiplet	2xPh
5.7	1H, broad	NH
5.17-5.08	2H, dd, $J=7.54\text{Hz}$	PhCH_2O
4.48-4.44	2H, multiplet	Pro α -CH, Phe α -CH
3.46	1H, multiplet	Pro-C4
3.27	2H, multiplet	CH_2OMe
3.22	3H, singlet	OMe

P41070GB1.2 - 50c Salts/Generic Salts I

40

2.99	2H, multiplet	PhCH ₂
2.63	1H, multiplet	CHB
2.59-2.23	4H, multiplet	Pro-C3, Pro-C2
1.60	4H, multiplet	CH ₂ CH ₂
1.20	12H, singlet	pinacol

The TRISOb should be checked by ¹³C NMR spectroscopy. ¹³C Signals should be observed as follows:-

δ400	Signal Pattern	Assignment
171	quaternary	O-CO-N
156	quaternary	CH-CO-N
136	quaternary	Ph
130-126	CH	aromatics
81.5	quaternary	CMe ₂
73	CH ₂	CH ₂ OMe
67.26	CH ₂	PhCH ₂ O
58.3	CH	Pro-αCH
57.94	CH ₃	OMe
54.46	CH	Ph-αCH
46.77	CH ₂	Pro-4-CH ₂
38.76	CH ₂	PhCH ₂ CH
27.84-27.4	2x CH ₂	CH ₂ CH ₂ CH ₂ OMe
25.23-24.9	4xCH ₃	pinacol, major isomer
24.07	CH ₂	Pro-3- CH ₂

5

Due to the presence of impurities other signals will be observed also.

3.3.2 HPLC Analysis

- 10 [note: a) tripeptide cannot be recovered from aqueous solution. b) Dipeptide elutes at solvent front and does not give a peak in this system]

- Column: Reverse phase C-18 ODS (octadecylsilane) 2.5μm, 150x4.6mm
- Flow: 1.5ml/min.
- Detection: UV at 225 nm

- 15• Injection volume: 0.02ml

- Solvent A: 20% MeCN in analytical grade water.

P41070GB1.2 - 50c Salts/Generic Salts I

41

- Solvent B: 55% MeCN in analytical grade water.
- Gradient: Linear from 20 to 90% mobile phase B over initial 15 minutes. Conditions maintained at 90% mobile phase B for a further 10 minutes. Linear to 100% B over 10mins, conditions maintained at 100% B for 5 mins then re-equilibrated to initial conditions.

5

Component	Rt (min)
Z-D-Phe-Pro-(S)-boroMpgOPinacol	16(+/-1)
Z-D-Phe-Pro-(R)-boroMpgOPinacol	17(+/-1)

Z-D-Phe-Pro-(R)-boroMpgOPinacol is the same chirality as Z-D-Phe-Pro-L-boroMpgOPinacol.

H. Cbz-D-Phe-Pro-BoroMpg-OH (TRI50c)

10

To a solution of TRI50b (mm 608) in acetone (1g/10ml), is added phenyl boronic acid (1.01 equivalent, mm 120) and the solution stirred by a mechanical stirrer. To the solution is slowly added ammonium hydroxide solution, (5%, pH adjusted to pH 9 by HCl, same volume as acetone). Some cloudiness may develop.

15

Hexane (equal volume to total acetone and ammonium hydroxide) is added and the solution stirred rapidly for four hours. Stirring is stopped and the hexane layer decanted (if an oil forms, this is kept with the aqueous layer by washing with a small volume of acetone). Hexane (same volume) is added, stirred for 10mins, decanted and repeated.

20

The aqueous layer is concentrated to about 1/3 volume by rotary evaporator with card-ice cold finger (water bath <35°C). Some oil may form on the side of the flask. The solution is then acidified (0.1N HCl) to pH 3 (care: do not acidify below pH 3), and extracted by EtOAc (2x same as original acetone volume). Sample can be concentrated without drying to give a foam, yield

25

~70%.

EXAMPLE 2 - ALTERNATIVE CONVERSION OF TRI50B TO TRI50C

1. Approximately 300 g of TRI50b were dissolved in approximately 2.5 L diethylether.
- 30 2. Approximately 54 ml diethanolamine were been added, the mixture was refluxed at 40 °C.
3. The precipitated product was removed, washed several times with diethylether and dried.
4. The dry product was dissolved in CHCl₃. Hydrochloric acid (pH 1) was added and the mixture was stirred approximately 1h at room temperature.
5. The organic layer was removed and washed with NH₄Cl solution.
- 35 6. The organic solvent was distilled off and the residual solid product was dried.

P41070GB1.2 - 50c Salts/Generic Salts I

42

Typical yield: Approximately 230 g

EXAMPLE 3 - PREPARATION OF LITHIUM SALT OF TRI50C

- 5 Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added LiOH as a 0.2M solution in distilled water (190ml). The resultant clear solution is stirred for 2 hours at room temperature and then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant oil/tacky liquid is redissolved in 500ml distilled water necessary with light warming for about 20 minutes. The solution is filtered through filter paper and evacuated to dryness, again with the temperature of the solution not exceeding 37°C. The resultant product is dried under vacuum overnight to normally yield a white brittle solid.

The salt was then dried under vacuum over silica to constant weight (72 h).

15

Yield 17.89g.

Microanalysis:

C % Found (Calc.)	H % Found (Calc.)	N % Found (Calc.)	B % Found (Calc.)	Metal % Found (Calc.)
57.14 (61.03)	6.60 (6.64)	7.34 (7.90)	2.07 (2.03)	Li 1.26 (1.31)

20

EXAMPLE 4 - UV/VISIBLE SPECTRA OF LITHIUM SALT OF TRI50C

- UV/Visible spectra were recorded in distilled water at 20°C from 190nm to 400nm. The salt gave λ_{max} at 210 and 258nm. The weight of the dried salt was then measured for the purposes of calculating the extinction coefficient. The λ_{max} at 258nm was used. The extinction coefficient was calculated using the formula:-

- $A = \epsilon cl$ where A is the absorbance
C is the concentration
l the path length of the UV cell
and ϵ is the extinction coefficient.

30

Extinction coefficient: 451

EXAMPLE 5 - AQUEOUS SOLUBILITY OF LITHIUM SALT OF TRI50C

To determine maximum aqueous solubility 25mg of the dried salt were shaken in water at 37°C, the sample filtered and the UV spectrum measured. The salt left a white residue of undissolved material. The lithium salt was comparatively soluble and so was redissolved at 50mg/ml in the same manner previously described.

Solubility when dissolved at 25mg/ml: 43mM (23 mg/ml).

Solubility when dissolved at 50mg/ml: 81mM (43 mg/ml).

EXAMPLE 6 - PREPARATION OF SODIUM SALT OF TRI50C

Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added NaOH as a 0.2M solution in distilled water (190ml). The resultant clear solution is stirred for 2 hours at room temperature and then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant oil/tacky liquid is redissolved in 500ml distilled water with light warming for about 15-20 minutes. The solution is filtered through filter paper and evacuated to dryness, again with the temperature of the solution not exceeding 37°C. The resultant product is dried under vacuum overnight to normally yield a white brittle solid. The product may be present as an oil or tacky solid due to residual water, in which case it is dissolved in ethyl acetate and evacuated to dryness to produce the product as a white solid.

The salt was then dried under vacuum over silica to constant weight (72 h).

Yield: Over 50%.

Microanalysis:

30

C % Found (Calc.)	H % Found (Calc.)	N % Found (Calc.)	B % Found (Calc.)	Metal % Found (Calc.)
59.93 (59.24)	6.47 (6.44)	7.31 (7.67)	1.91 (1.98)	Na 3.81 (4.20)

EXAMPLE 7 - UV/VISIBLE SPECTRA OF SODIUM SALT OF TRI50C

UV/Visible spectra were recorded in distilled water at 20°C from 190nm to 400nm. The salt gave λ_{max} at 210 and 258nm. The weight of the dried salt was then measured for the purposes of

P41070GB1.2 - 50c Salts/Generic Salts I

45

54.84 (57.55)	6.25 (6.26)	7.02 (7.45)	2.01 (1.92)	K 4.29 (6.94)
------------------	----------------	----------------	----------------	------------------

EXAMPLE 10 – UV/VISIBLE SPECTRA OF POTASSIUM SALT OF TRI50C

- 5 UV/Visible spectra were recorded in distilled water at 20°C from 190nm to 400nm. TRI50C and the salt gave λ_{max} at 210 and 258nm. The weight of the dried salt was then measured for the purposes of calculating the extinction coefficient. The λ_{max} at 258nm was used. The extinction coefficient was calculated using the formula:-
- 10 $A = \epsilon cl$ where A is the absorbance
 C is the concentration
 l the path length of the UV cell
and ϵ is the extinction coefficient.
- 15 Extinction coefficient: 438.

EXAMPLE 11 – AQUEOUS SOLUBILITY OF POTASSIUM SALT OF TRI50C

- 20 To determine maximum aqueous solubility 25mg of the dried salt were shaken in water at 37°C, the sample filtered and the UV spectrum measured. The salt left a white residue of undissolved material.

Solubility when dissolved at 25mg/ml: 29mM (16 mg/ml).

25 EXAMPLE 12 – PREPARATION OF CALCIUM SALT OF TRI50C

- 30 Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added $\text{Ca}(\text{OH})_2$ as a 0.1M solution in distilled water (190ml). The resultant clear solution is stirred for 2 hours at room temperature and then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant product is a white brittle solid.

The salt was then dried under vacuum over silica to constant weight (72 h).

- 35 Yield: 17.69g.

Microanalysis:

P41070G81.2 - 50c Salts/Generic Salts I

46

C % Found (Calc.)	H % Found (Calc.)	N % Found (Calc.)	B % Found (Calc.)	Metal % Found (Calc.)
55.08 (59.27)	6.43 (6.48)	7.08 (7.71)	2.01 (1.99)	Ca 3.65 (3.68)

EXAMPLE 13 - UV/VISIBLE SPECTRA OF CALCIUM SALT OF TRI50C

5

UV/Visible spectra were recorded in distilled water at 20°C from 190nm to 400nm. TRI50C and the salt gave λ_{\max} at 210 and 258nm. The weight of the dried salt was then measured for the purposes of calculating the extinction coefficient. The λ_{\max} at 258nm was used. The extinction coefficient was calculated using the formula:-

10

$A = scl$ where A is the absorbance

C is the concentration

l the path length of the UV cell

and s is the extinction coefficient.

15

Extinction coefficient: 955.

EXAMPLE 14 - AQUEOUS SOLUBILITY OF CALCIUM SALT OF TRI50C

20 To determine maximum aqueous solubility 25mg of the dried salt were shaken in water at 37°C, the sample filtered and the UV spectrum measured. The salt left a white residue of undissolved material.

Solubility when dissolved at 25mg/ml: 5mM (5 mg/ml).

25

EXAMPLE 15 - PREPARATION OF ARGININE SALT OF TRI50C

30 Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added arginine as a 0.2M solution in distilled water (190ml). The resultant clear solution is stirred for 2 hours at room temperature and then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant oil/tacky liquid is redissolved in 2L distilled water with warming to 37°C for 2 hours. The solution is filtered through filter paper and evacuated to dryness, again with the temperature of the

P41070GB1.2 - 50c Salts/Generic Salts I

47

solution not exceeding 37°C. The resultant product is dried under vacuum overnight to normally yield a white brittle solid.

The salt was then dried under vacuum over silica to constant weight (72 h).

5

Yield: 10.54g.

Microanalysis:

C % Found (Calc.)	H % Found (Calc.)	N % Found (Calc.)	B % Found (Calc.)
52.47 (56.65)	7.12 (7.20)	15.25 (14.01)	1.52 (1.54)

10

EXAMPLE 16 – UV/VISIBLE SPECTRA OF ARGININE SALT OF TRI50C

UV/Visible spectra were recorded in distilled water at 20°C from 190nm to 400nm. TRI50C and the salt gave λ_{\max} at 210 and 258nm. The weight of the dried salt was then measured for the purposes of calculating the extinction coefficient. The λ_{\max} at 258nm was used. The extinction coefficient was calculated using the formula:-

$A = \epsilon cl$ where A is the absorbance
 C is the concentration
 l the path length of the UV cell
and ϵ is the extinction coefficient.

20

Extinction coefficient: 406.

25

EXAMPLE 17 – AQUEOUS SOLUBILITY OF ARGININE SALT OF TRI50C

To determine maximum aqueous solubility 25mg of the dried salt were shaken in water at 37°C, the sample filtered and the UV spectrum measured. The salt left a white residue of undissolved material.

30

Solubility when dissolved at 25mg/ml: 14mM (10 mg/ml).

EXAMPLE 18 – PREPARATION OF LYSINE SALT OF TRI50C

P41070GB1.2 - 50c Salts/Generic Salts I

48

5 Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added L-lysine as a 0.2M solution in distilled water (190ml). The resultant clear solution is stirred for 2 hours at room temperature and then
 10 evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant oil/tacky liquid is redissolved in 3L distilled water with warming to 37°C for 2 hours. The solution is filtered through filter paper and evacuated to dryness, again with the temperature of the solution not exceeding 37°C. The resultant product is dried under vacuum overnight to normally yield a white brittle solid. The product may be present as an oil or tacky solid (due to residual water), in which case it is then dissolved in ethyl acetate and evacuated to dryness to produce the product as a white solid.

The salt was then dried under vacuum over silica to constant weight (72 h).

15 Yield: 17.89.

Microanalysis:

C % Found (Calc.)	H % Found (Calc.)	N % Found (Calc.)	B % Found (Calc.)
57.03 (59.11)	7.43 (7.36)	10.50 (10.44)	1.72 (1.61)

20

EXAMPLE 19 – UV/VISIBLE SPECTRA OF LYSINE SALT OF TRI50C

UV/Visible spectra were recorded in distilled water at 20°C from 190nm to 400nm. TRI50C and the salt gave λ_{max} at 210 and 258nm. The weight of the dried salt was then measured for the
 25 purposes of calculating the extinction coefficient. The λ_{max} at 258nm was used. The extinction coefficient was calculated using the formula:-

$A = \epsilon cl$ where A is the absorbance

C is the concentration

30

l the path length of the UV cell

and ϵ is the extinction coefficient.

Extinction coefficient: 437.

35

EXAMPLE 20 – AQUEOUS SOLUBILITY OF LYSINE SALT OF TRI50C

P41070GB1.2 - 50c Salts/Generic Salts I

49

To determine maximum aqueous solubility 25mg of the dried salt were shaken in water at 37°C, the sample filtered and the UV spectrum measured. The salt left a white residue of undissolved material.

5

Solubility when dissolved at 25mg/ml: 13mM (8.6 mg/ml).

EXAMPLE 21 - PREPARATION OF N-METHYL-D-GLUCAMINE SALT OF TRI50C

- 10 Cbz-Phe-Pro-BoroMpg-OH (20.00g, 38.1mM) is dissolved in acetonitrile (200ml) with stirring at room temperature. To this solution is added N-methyl-D-glucamine as a 0.2M solution in distilled water (190ml). The resultant clear solution is stirred for 2 hours at room temperature and then evacuated to dryness under vacuum with its temperature not exceeding 37°C. The resultant oil/tacky liquid is redissolved in 500ml distilled water with light warming for about 20
- 15 minutes. The solution is filtered through filter paper and evacuated to dryness, again with the temperature of the solution not exceeding 37°C, or freeze dried. The resultant product is dried under vacuum overnight to normally yield a white brittle solid.

The salt was then dried under vacuum over silica to constant weight (72 h).

20

Yield: 21.31g.

Microanalysis:

C % Found (Calc.)	H % Found (Calc.)	N % Found (Calc.)	B % Found (Calc.)
56.67 (56.67)	7.28 (7.41)	7.74 (7.77)	1.63 (1.50)

25

EXAMPLE 22 - UV/VISIBLE SPECTRA OF N-METHYL-D-GLUCAMINE SALT OF TRI50C

- UV/Visible spectra were recorded in distilled water at 20°C from 190nm to 400nm. TRI50C and the salt gave λ_{\max} at 210 and 258nm. The weight of the dried salt was then measured for the purposes of calculating the extinction coefficient. The λ_{\max} at 258nm was used. The extinction coefficient was calculated using the formula:-
- 30

$A = \epsilon c l$ where A is the absorbance

35

C is the concentration

P41070GB1.2 - 50c Salts/Generic Salts I

50

l the path length of the UV cell
and ϵ is the extinction coefficient.

Extinction coefficient: 433.

5

EXAMPLE 23 - AQUEOUS SOLUBILITY OF N-METHYL-D-GLUCAMINE SALT OF TRI50C

To determine maximum aqueous solubility 25mg of the dried salt were shaken in water at 37°C, the sample filtered and the UV spectrum measured. The salt was observed to fully dissolve. The
10 salt was comparatively soluble and so was redissolved at 50mg/ml in the same manner previously described.

Solubility when dissolved at 25mg/ml: 35mM (25 mg/ml).

Solubility when dissolved at 50mg/ml: 70mM (50 mg/ml).

15

EXAMPLE 24 - ALTERNATIVE PREPARATION OF ARGININE SALT OF TRI50C

The arginine salt is formed simply by adding a slight molar excess of L-arginine to a solution of
20 0.2-0.3mmol of TRI50c in 10ml of ethyl acetate. The solvent is evaporated after one hour, and the residue is triturated twice with hexane to remove excess arginine.

EXAMPLE 25 - SOLUBILITY OF TRI50C

The UV/visible spectra of TRI50c and its solubility were obtained as described above in relation
25 to the salts. The solubility of TRI50c when dissolved at 50mg/ml was 8mM (4mg/ml).

EXAMPLE 26 - SEPARATION OF DIASTEREOMERS

The R-Mpg and S-Mpg isomers of TRI50b and TRI50c are separated chromatographically as
30 summarised below.

A solution of 5gm/ml of TRI50b in acetonitrile is prepared and 10 μ L is injected to a Lichrosphere™ cyano column and eluted with a gradient of n-hexane and tetrahydrofuran with monitoring at 206nm. Analysis of the UV chromatogram indicates TRI50b isomer I ('R'
35 configuration at α -aminoboronate centre) elutes at (retention time) Rt 11.1 minutes; TRI50b isomer II ('S' configuration at α -aminoboronate centre) elutes at Rt 13.7minutes.

P41070GB1.2 - 50c Salts/Generic Salts I

51

Following the same procedure, TRI50c isomer I ('R' configuration at α -aminoboronate centre) elutes at (retention time) Rt 21.2 minutes; TRI50b isomer II ('S' configuration at α -aminoboronate centre) elutes at Rt 22.2 minutes.

5 *Conditions:*

Column: Licrosphere Cyano Merck.4.6 x 250mm, 5 μ .

Solvent A : n-Hexane

Solvent B THF

Gradient 0-100% B over 25 minutes

10

Monitor UV at 206nm

Sample concentration 5mg/ml.

The results are shown in the chromatogram of Fig 8.

15

The above microanalytical data show C and N amounts below calculated, suggesting the samples might have contained unremoved water.

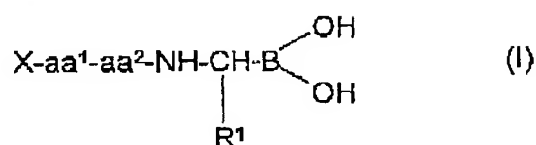
Advantageously, at least preferred products of the invention have adequate absorption and
20 bioavailability. For commercial utility, a product having less good solubility may be selected by virtue of a superior overall combination of properties.

P41070GB1.2 - 50c Salts/Generic Salts 1

52

CLAIMS

1. A salt of a peptide boronic acid of formula (I):



5

where:

X is H (to form NH₂) or an amino-protecting group;

- 10 aa¹ is Phe, Dpa or a wholly or partially hydrogenated analogue thereof;

aa² is an imino acid having from 4 to 6 ring members;

- 15 R¹ is a group of the formula -(CH₂)_m-W, where m is 2, 3 or 4 and W is -OH, -OMe, -OEt or halogen (F, Cl, Br or I).

2. A salt of claim 1 wherein aa¹ is selected from Dpa, Phe, Dcha and Cha.

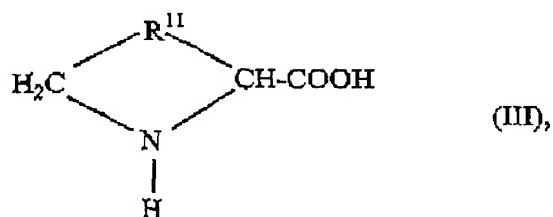
3. A salt of claim 1 or claim 2 wherein aa¹ is of R-configuration.

20

4. A salt of claim 3 wherein aa¹ is (R)-Phe (that is, D-Phe) or (R)-Dpa (that is, D-Dpa).

5. A salt of claim 3 wherein aa¹ is (R)-Phe.

- 25 6. A salt of any of claims 1 to 5 wherein aa² is a residue of an imino acid of formula (III)

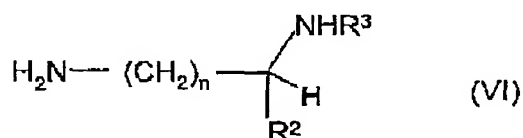


P41070GB1.2 - 50c Salts/Generic Salts I

53

where R^{11} is $-\text{CH}_2-$, CH_2-CH_2- , $-\text{S}-\text{CH}_2-$, $-\text{S}-\text{C}(\text{CH}_3)_2-$ or $-\text{CH}_2-\text{CH}_2-\text{CH}_2-$, which group, when the ring is 5- or 6- membered, is optionally substituted at one or more $-\text{CH}_2-$ groups by from 1 to 3 C_1-C_3 alkyl groups.

- 5 7. A salt of claim 6 wherein aa^2 is of S-configuration.
8. A salt of claim 6 wherein aa^2 is a natural proline residue.
9. A salt of claim 1, wherein aa^1-aa^2 is (R)-Phe-(S)-Pro (that is, D-Phe-L-Pro).
- 10 10. A salt of any of claims 1 to 9 wherein R^1 is 2-bromoethyl, 2-chloroethyl, 2-methoxyethyl, 3-bromopropyl, 3-chloropropyl or 3-methoxypropyl.
11. A salt of any of claims 1 to 9 wherein R^1 is 3-methoxypropyl.
- 15 12. A salt of any of claims 1 to 11 wherein X is benzyloxycarbonyl.
13. A salt of claim 1 which is a salt of a compound of formula (VIII):
- 20 X-(R)-Phe-(S)-Pro-(R)-Mpg-B(OH)₂ (VIII).
14. A salt of any of claims 1 to 13 which is a salt of the peptide boronic acid with an alkali metal, an alkaline earth metal, a Group III metal or a strongly basic organic nitrogen-containing compound.
- 25 15. A salt of claim 14 wherein the strongly basic organic nitrogen-containing compound is a guanidine, a guanidine analogue or an amine.
16. A salt of any of claims 1 to 13 which is a salt of the peptide boronic acid with an alkali
- 30 metal, an aminosugar, a guanidine or an amine of formula (VI):

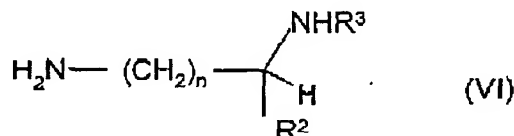


where n is from 1 to 6, R^2 is H, carboxylate or derivatised carboxylate, R^3 is H, C_1-C_4 alkyl or a residue of a natural or unnatural amino acid.

P41070GB1.2 - 50c Salts/Generic Salts I

54

17. A salt of claim 16 which is a salt of the peptide boronic acid with an alkali metal or an amino sugar.
- 5 18. A salt of claim 17 wherein the alkali metal is sodium or lithium.
19. A salt of claim 17 or claim 18 wherein the amino sugar is a glucamine.
20. A salt of claim 19 wherein the glucamine is N-methyl-D-glucamine.
- 10 21. A salt of any preceding claim which is an acid salt (that is, wherein one B-OH group remains protonated).
22. A salt of any of claims 1 to 20 wherein the salt consists essentially of an acid salt (that is, wherein one B-OH group remains protonated).
- 15 23. A salt of any of claims 1 to 22 wherein the salt comprises a boronate ion derived from the peptide boronic acid and a counterion and wherein the salt consists essentially of a salt having a single type of counterion.
- 20 24. A salt of any of claims 1 to 13 which is a salt of the peptide boronic acid with a guanidine or with an amine of formula (VI):



25 where n is from 1 to 6, R^2 is H, carboxylate or derivatised carboxylate, R^3 is H, $\text{C}_1\text{-C}_4$ alkyl or a residue of a natural or unnatural amino acid.

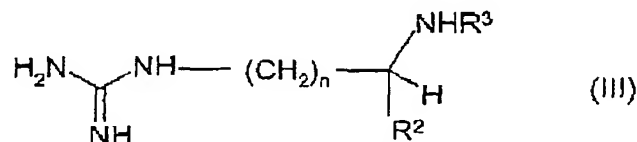
25. A salt of claim 24 which is a guanidine salt of the peptide boronic acid.
26. A salt of claim 25 which is a salt of the peptide boronic acid with L-arginine or an L-arginine analogue.
- 30 27. A salt of claim 26 wherein the L-arginine analogue is D-arginine, or the D- or L- isomers of homoarginine, agmatine [(4-aminobutyl) guanidine], NG-nitro-L-arginine methyl ester, or a 2-amino pyrimidines.

35

P41070GB1.2 - 50c Salts/Generic Salts I

55

28. A salt of claim 25 which is a salt of the peptide boronic acid with a guanidine of formula (III)



where n is from 1 to 6, R² is H, carboxylate or derivatised carboxylate, R³ is H, C₁-C₄ alkyl or a residue of a natural or unnatural amino acid.

5

29. A salt of claim 28, wherein n is 2, 3 or 4.

30. A salt of claim 28 or claim 29 where the derivatised carboxylate forms a C₁-C₄ alkyl ester or amide.

10

31. A salt of any of claims 28 to 30 wherein the compound of formula (III) is of L-configuration.

32. A salt of claim 25 which is an L-arginine salt of the peptide boronic acid.

15

33. A salt of claim 24 which is a salt of the peptide boronic acid with an amine of formula (VI).

34. A salt of claim 33, wherein n is 2, 3 or 4.

20

35. A salt of claim 33 or claim 34 where the derivatised carboxylate forms a C₁-C₄ alkyl ester or amide.

36. A salt of any of claims 33 to 35 wherein the compound of formula (III) is of L-configuration.

25

37. A salt of claim 33 which is an L-lysine salt of the peptide boronic acid.

38. A salt of any of claims 24 to 37 which is an acid salt (that is, wherein one B-OH group remains protonated).

30

39. A salt of any of claims 24 to 38 wherein the salt consists essentially of acid salt (that is, wherein one B-OH group remains protonated).

P41070GB1.2 - 50c Salts/Generic Salts I

56

40. A salt of any of claims 24 to 39 wherein the salt comprises a boronate ion derived from the peptide boronic acid and a counterion and wherein the salt consists essentially of a salt having a single type of counterion.
- 5 41. A salt of any of claims 1 to 13 which is an alkali metal salt of the peptide boronic acid.
42. A salt of claim 41 wherein the alkali metal is potassium.
43. A salt of claim 42 wherein the alkali metal is sodium or lithium.
- 10 44. A salt of claim 43 wherein the alkali metal is sodium.
45. A salt of claim 43 wherein the alkali metal is lithium.
- 15 46. A salt of any of claims 41 to 45 which is an acid salt (that is, wherein one B-OH group remains protonated).
47. A salt of any of claims 41 to 45 wherein the salt consists essentially of acid salt (that is, wherein one B-OH group remains protonated).
- 20 48. A salt of any of claims 41 to 47 wherein the salt comprises a boronate ion derived from the peptide boronic acid and a counterion and wherein the salt consists essentially of a salt having a single type of counterion.
- 25 49. A salt of any of claims 1 to 13 which is an aminosugar salt of the peptide boronic acid.
50. A salt of claim 49 wherein the aminosugar is a ring-opened sugar.
51. A salt of claim 50 wherein the aminosugar is a glucamine.
- 30 52. A salt of claim 49 wherein the aminosugar is a cyclic aminosugar.
53. A salt of any of claims 49 to 52 wherein the aminosugar is N-unsubstituted.
- 35 54. A salt of any of claims 49 to 52 wherein the aminosugar is N-substituted by one or two substituents.
55. A salt of claim 54 wherein the or each substituent is a hydrocarbyl group.

P41070GB1.2 - 50c Salts/Generic Salts I

57

56. A salt of claim 54 wherein the or each substituent is selected from the group consisting of alkyl and aryl moieties.

57. A salt of claim 56 wherein the or each substituent is selected from the group consisting of C₁, C₂, C₃, C₄, C₅, C₆, C₇ and C₈ alkyl groups

58. A salt of any of claims 54 to 57 wherein there is a single N-substituent.

59. A salt of claim 49 wherein the glucamine is N-methyl-D-glucamine.

60. A salt of any of claims 49 to 59 which is an acid salt (that is, wherein one B-OH group remains protonated).

61. A salt of any of claims 49 to 59 wherein the salt consists essentially of acid salt (that is, wherein one B-OH group remains protonated).

62. A salt of any of claims 49 to 61 wherein the salt comprises a boronate ion derived from the peptide boronic acid and a counterion and wherein the salt consists essentially of a salt having a single type of counterion.

63. A product obtainable by (having the characteristics of a product obtained by) reaction of a peptide boronic acid as defined in any of claims 1 to 13 and a base capable of forming a salt therewith.

64. A product obtainable by (having the characteristics of a product obtained by) reaction of a peptide boronic acid as defined in any of claims 1 to 13 and a base selected from the group consisting of a hydroxide of a metal as recited in any of claims 14 or 16 to 18 or with an organic nitrogen-containing compound whose pK_b is 7 or more.

65. A product obtainable by (having the characteristics of a product obtained by) reaction of a peptide boronic acid as defined by any of claims 1 to 13 and a base selected from the group consisting of an organic nitrogen-containing compounds as recited in any of claims 24 to 37 or 49 to 59.

66. A product of any of claims 63 to 65 wherein the reaction comprises combining a solution of the peptide boronic acid in a water-miscible organic solvent with an aqueous solution of the base, allowing the acid and the base to react at ambient temperature (e.g. at a temperature of from 15 to 25°C), evacuating the reaction mixture to dryness, redissolving the salt in water,

P41070GB1.2 - 50c Salts/Generic Salts I

58

filtering the resulting solution and drying it, and, if required, removing at least a portion of the residual water by further redissolution in ethyl acetate followed by evaporating to dryness.

67. A product of claim 66 wherein the acid and the base are allowed to react for at least one
5 hour.

68. A product of claim 66 or claim 67 wherein the water-miscible organic solvent is
10 acetonitrile or an alcohol, e.g. ethanol, methanol, a propanol, especially iso-propanol, or another
alkanol, or a mixture of alcohols.

69. A method for drying a peptide boronic acid salt, comprising dissolving it in ethyl acetate
15 and then evaporating the resultant solution to dryness.

70. A pharmaceutical formulation in oral dosage form comprising a salt of any of claims 1 to
15 62 or a product of any of claims 63 to 68 and a pharmaceutically acceptable diluent, excipient or
carrier.

71. A pharmaceutical formulation of claim 70 which is adapted to release the salt in the
20 duodenum.

72. A pharmaceutical composition of claim 71 which is enterically coated.

73. A method of inhibiting thrombin in the treatment of disease comprising orally
25 administering to a mammal a therapeutically effective amount of an active agent selected from
the group consisting of salts of any of claims 1 to 62 and products of any of claims 63 to 68.

74. A method of claim 73 wherein the active agent is in a formulation adapted to release the
active agent in the duodenum.

30 75. The use of a salt of any of claims 1 to 62 or a product of any of claims 63 to 68 for the
manufacture of an oral medicament for treating thrombosis.

76. The use of a peptide boronic acid of formula (I) as defined in any of claims 1 to 13 as an
35 intermediate to make a salt of any of claims 1 to 62 or a product of any of claims 63 to 68.

77. A method of preparing a salt of any of claims 1 to 62 or a product of any of claims 63 to
68, comprising contacting a peptide boronic acid of formula (I) as defined in claim 1 with a base
capable of making such a salt.

P41070GB1.2 - 50c Salts/Generic Salts I

59

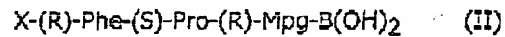
78. A peptide boronic acid of formula (I) as defined in any of claims 1 to 13 when of GLP or GMP quality, or when in compliance with GLP (good laboratory practice) or GMP (good manufacturing practice).

5 79. A composition of matter which is sterile or acceptable for pharmaceutical use, or both, and comprises a peptide boronic acid of formula (I) as defined in any of claims 1 to 13.

80. A composition of matter of claim 79 which is in particulate form.

10 81. A composition of claim 79 which is in the form of a liquid solution or dispersion.

82. An isolated compound which is a peptide boronic acid of formula (II):



15

wherein X is H (to form NH_2) or an amino-protecting group.

83. A compound of claim 82 wherein X is benzyloxycarbonyl.

20 84. A particulate composition comprising a peptide boronic acid of formula (II) as defined in claim 82 or claim 83.

85. A composition of claim 84 consisting predominantly of the peptide boronic acid.

25 86. A composition of claim 85 wherein the peptide boronic acid forms at least 75% by weight of the composition.

87. A composition of claim 86 wherein the peptide boronic acid forms at least 85% by weight of the composition.

30

88. A composition of claim 87 wherein the peptide boronic acid forms at least 95% by weight of the composition.

89. A composition of any of claims 84 to 88 which is sterile.

35

90. A composition of any of claims 84 to 89 wherein the peptide boronic acid is in finely divided form.

P41070GB1.2 - 50c Salts/Generic Salts I

60

91. A liquid composition consisting of, or consisting essentially of, a peptide boronic acid of formula (I) as defined in any of claims 1 to 13 and liquid vehicle in which it is dissolved or suspended.

5 92. A liquid composition of claim 91 wherein the liquid vehicle is an aqueous medium, e.g. water.

93. A liquid composition of claim 91 wherein the liquid vehicle is an alcohol, for example methanol, ethanol, isopropanol or another propanol, another alkanol or a mixture of the
10 foregoing.

94. A liquid composition of any of claims 91 to 93 which is sterile.

